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PURPOSES

The main purpose of the Physiological Reviews is to furnish a means whereby those interested in the physiological sciences may keep in touch with contemporary research. The literature, as every worker knows, is so extensive and scattered that even the specialist may fail to maintain contact with the advance along different lines of his subject. The obvious method of meeting such a situation is to provide articles from time to time in which the more recent literature is compared and summarized. The abstract journals render valuable assistance by condensing and classifying the literature of individual papers, but their function does not extend to a comparative analysis of results and methods. Publications such as the Ergebnisse der Physiologie, the Harvey Lectures, etc., that attempt this latter task, have been so helpful as to encourage the belief that a further enlargement of such agencies will be welcomed by all workers. It is proposed, therefore, to establish a journal in which there will be published a series of short but comprehensive articles dealing with the recent literature in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology.

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PHYSIOLOGICAL ACTION CURRENTS IN THE PHRENIC NERVE. AN APPLICATION OF THE THERMIONIC VACUUM TUBE TO NERVE PHYSIOLOGY[1]

H. S. GASSER AND H. S. NEWCOMER

From the Laboratory of Physiology of the Washington University Medical School and the Laboratory of the Department for Mental and Nervous Diseases of the Pennsylvania Hospital

Received for publication April 9, 1921

Many experimenters who have had experience with the recording of the action currents of nerve have felt the need of more sensitive apparatus than has been available. The development of the three electrode vacuum tube has suggested independently to a number of physiologists that this instrument might be of great value in the elucidation of problems in electro-physiology. It was first suggested to one of the present authors in the spring of 1919 in a conversation with Mr. A. S. Langsdorf. Since we have been working on this problem three papers have appeared on the application of the vacuum tube to physiology. Höber (1) repeated the older observations on muscle made by Bernstein and Schoenlein, and by Wedenski with a telephone, bringing to his aid a vacuum tube cascade. Little description of his instrument is given. From the diagram it appears to be a three tube transformer coupled cascade connected to input and output also by transformers. The three filaments were operated from one battery as were also the three plate circuits. No graphic methods were used; the author states, however, that the apparatus was noisy.

[1] Both amplifiers used were built by Doctor Newcomer at the Pennsylvania Hospital. The experiments were jointly carried out at the Washington University.

EKG. 10-24-21

Forbes and Thacher (2) have described a very serviceable arrangement by which the action current of nerve may be amplified and recorded with the string galvanometer. For this they used one tube with the output connected to the galvanometer through a condenser. They also made a very detailed study of their circuit in the search for the conditions giving optimum amplification. Recently Daly and Shellshear (3) have described an apparatus in which the tubes were each placed in one arm of a Wheatstone bridge. They found three valves in cascade were necessary to give convenient amplification. Their apparatus has the advantage of aperiodicity, but is not, however, in its present form, of value for nerve studies on account of the large amount of adventitious vibration appearing in their records.

The apparatus used in our first experiments (June, 1920) consisted in an alternative one, two or three stage amplifier, resistance coupled when used in cascade. The input could be connected either directly or

Fig. 1. Action currents from the phrenic nerve showing an admixture of "noise." The record was made with two tubes and both the input and output transformers. Time, $\frac{1}{5}$ second. Magnification in all records 500 diameters.

through a transformer; the output could be connected to the galvanometer either through a transformer or a condenser. One 6 volt storage cell served for all three filaments and one 150 volt dry cell battery operated all three plate circuits. As the result of the loss of strings in attempts to use the condenser output we confined our experiments at this time to the transformer. In the light of our subsequent experience these accidents seem in all probability to have been due to a leak between the string mounting and the ground.

As we were interested primarily in the recording of physiological action currents, the phrenic nerve was used as a test object. Good amplification was obtained with one or two tubes. Three tube amplification seemed to be more than was practicable. Figure 1 shows the action current obtained from the phrenic nerve during the latter part of inspiration and the beginning of expiration. Two tubes and the transformer input and output connections were used. It will be seen

from the record that the string was not quiet during the respiratory pause but shows a double rhythm, one of 63, the other of 380 per second. This rhythm is continuous and may be traced into the records of the action current.

As the result of these experiments it was apparent that the problem of major importance was to so change the design that the galvanometer string would be quiet when there were no input connections. The apparatus was accordingly rebuilt as will be described, and proved to be more satisfactory.

The nature of the central innervation of muscle was selected as a good type of investigation that could be improved by directing the attention to the action currents of the nerve as well as to those of the muscle. Most of the numerous investigations of this subject have been made on the action currents of muscle because means have been lacking for the ready study of nerve activated from the central nervous system.

Piper (4) led off the action currents from the forearm to the string galvanometer and obtained a rhythmic oscillation of the string at the rate of about 50 per second. He interpreted this as the rate of central innervation. This interpretation met with considerable opposition as it was known from previous work, and has been confirmed by more recent work, that the action currents of muscle do not necessarily follow the stimulus when applied either directly or indirectly through the nerve (5), (6), (7). Wedenski, who studied muscle by means of a telephone, found that the sound followed the rate of stimulation up to a limit and then the muscle responded with a lower tone. When the rate of stimulation was very fast the "bruit" in the telephone was very similar to the sound produced by voluntary innervation; he therefore ventured the opinion that the innervation rate of muscle may be very much higher than that obtained from the muscle.

Wedenski's view was supported by Buchanan and by Garten. It was found that a rhythmic response was obtained from muscle indistinguishable from that occurring in voluntary innervation, not only from stimuli of high frequency but from the tetanus producing stimulus which occurs from opening or closing a strong constant current applied to nerve or muscle. Von Kries (8) had previously shown that a prolonged stimulus from a rheotome gave both a longer contraction and action current than results from an induction shock and concluded that the natural stimulus to muscle may vary in duration as well as in strength and rate. These findings were confirmed by Hoffmann (9)

who, however, found as the result of more delicate methods that the muscle action current consisted, depending on the duration of the stimulus, of from one to five waves and that the contraction was therefore a short tetanus. In the light of experiments by Dittler and Oinuma (10) and Beritoff (11), the appearance of a rhythm in the action current, depending upon the nature and the condition of the tissue and partially independent of the rate of stimulation, when the tissue is stimulated by a rapidly oscillating or strong constant current, may be attributable to the refractory period of the tissue. In a later work Garten (12) found that the oscillatory action current of muscle produced by a closing tetanus is also present in the nerve. He then expressed the opinion that the nerve rhythm is of the same order of magnitude as the muscle rhythm and thereby allows the possibility that if the nerve rhythm is determined by the central nervous system, the muscle rhythm is centrally controlled.

The sum of the experiments on the action currents of muscle clearly shows that when a given rhythm is obtained by leading from muscle in voluntary activity to a galvanometer, no conclusion is possible as to whether or not this rhythm is also the rhythm in its nerve. It is also equally true that the intrinsic period (Eigenperiode) of the muscle is not revealed.

The question can only be settled by an examination of both the muscle and its nerve, preferably simultaneously. This was done by Dittler (13) in the case of the diaphragm. Action currents were taken from the diaphragm of the rabbit and cat and compared with those obtained from the phrenic nerve. In the latter case he was sufficiently successful on connecting the cut end and side of the nerve to the string galvanometer to obtain oscillations whose rate could be determined for a part of the respiratory cycle. The nerve rate was very close to that of the muscle. In a later investigation, Dittler and Garten (14) recorded with two large string galvanometers the action currents of the dog's phrenic simultaneously with the action currents of the corresponding side of the diaphragm. Under these conditions where the action currents were necessarily smaller than those obtained by a lead from the cut end and side of the nerve, they obtained small pointlike deviations in the string picture during inspiration, recognizable with a lens. In the more successful experiments they believed they were able to identify corresponding waves in the records of the phrenic and diaphragm but state that it would be desirable for a more exact judgment to obtain larger and therefore more convincing curves of the diphasic action current.

METHODS

The experiments were done on dogs under a morphine and ether anesthesia. A wide exposure of the phrenic nerve usually on the right side was made and the nerve dissected free from the vena cava, surrounding connective tissue and fat, then severed close to the diaphragm. This gives a long stretch of nerve which belongs entirely to one muscle. The nerve was fastened through three non-polarizable electrodes containing wool yarn that could be tied about the nerve. The third electrode was not filled with zinc sulphate but served merely as a support to prevent movements from being translated to the other two. All the electrodes were supported on a stand, free from the operating table to prevent vibration during respiration. The distal electrode was connected with the cut end of the nerve and the ground. The proximal was connected to the side of the nerve and to the grid of the first tube. By means of a double throw switch the preparation could be connected to the galvanometer either directly or through the amplifier. The artificial ventilation necessary on account of the open thorax was maintained to the point of apnea. The records were obtained during periods of temporary suspension of the artificial respiration.

For a discussion of amplifier design the reader is referred to a chapter on that subject by Van der Bijl (15). Figure 2 is a diagram of the amplifier used in these experiments. The essential parts were all manufactured by the Western Electric Company. The original design used in June, 1920, was adapted at their suggestion from that of one of their standard sets and Mr. R. E. Bitner of their Research Branch has since given us valuable suggestions, the most important of which was to reduce the adventitious vibrations by using separate batteries for each tube. The input is connected to the grid circuit either directly or through a transformer. In the first instance the grid circuit is shunted with a resistance of 2 million ohms, in the latter with one of 100,000 ohms. The transformer has four taps permitting one to vary with the switch, S_1, the input impedence of the circuit to an optimum. Each tube is operated by a separate plate battery of 150 to 160 volts consisting of no. 734 Eveready dry cells. The filaments are operated separately by three storage batteries of 6 volts each. Two resistances of 500,000 ohms each are inserted between the second and third grids and the ground. These serve to maintain the grids negative to the filaments because of the potential drop in the filament resistances of the filament circuits. In the plate circuit of each tube is inserted a

resistance of 200,000 ohms separately shielded. The second and third grids are protected from the potentials of the preceding plates by the insertion of condensers each having a capacity of 2 microfarads. Their resistance is 4 billion ohms. The tube and the plate battery act as a generator of alternating potential because of variations in the tube resistance. This potential is applied across the circuit consisting of the coupling condenser and the 500,000 ohm resistance and thus varies the potential of the next grid. The alternating current output of

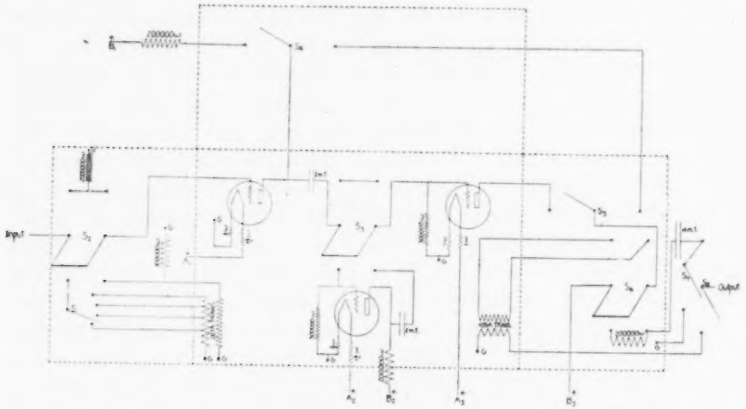

Fig. 2. The dotted lines represent the outlines of the metallic shield, the sides and back being thrown open to show the wiring. The points G indicate the grounding of a wire to the shield. The three A batteries of 6 volts each and the three B batteries of 150 volts each have their negative poles all connected to the shield. The switches S_2, S_6 and S_3 permit changing from a condenser to a transformer circuit. S_7 is a shunting switch to charge the output condenser. S_3 changes the set from a two to a three stage amplifier. S_4 and S_5 change the set from a two stage to a one stage amplifier. S_1 changes the optimum input impedance for the input transformer from 20 to 120,000 ohms.

the last tube is shunted across the 200,000 ohm resistance through a 14 microfarad condenser and the galvanometer string. The condenser has a resistance of 500 million ohms. In a few experiments a 34 microfarad condenser was used. As an alternative it is possible by the use of the double throw switches, S_6 and S_7, to connect the output of the last tube to the primary and the string to the secondary of a transformer. The optimum output impedence of this transformer is 500 ohms, and the input impedence matches that of the tube.

The vacuum tubes are the Western Electric type V. They are designed for use with a filament current of 1.3 amperes, a grid voltage of −1.5, a normal plate voltage of 100, and a maximum input voltage of 2. The value of the amplification constant μ is 28. The plate current under the conditions of our circuit is 0.45 milliampere. The d.c. plate-filament resistance is therefore 155,000 ohms and the a.c. resistance 77,500 ohms.

The actual wiring of the amplifier is nearly like that of the diagram. The whole is within the indicated shield consisting of a wooden box with a top and bottom of $\frac{1}{8}$ inch steel and a lining around the sides of $\frac{1}{16}$ inch lead. The box is padded on the inside with $\frac{3}{4}$ inch wool felt. The three tubes are screwed to a pine board and the latter wedged about with rubber bath sponges. Switch S_3 makes it easy to cut out one tube and obtain a two stage amplifier using the two outside tubes. In practice we never used more than the two tubes. Switches S_4 and S_5 were inserted subsequently in order to use one tube only. The rather heavy amplifier box with hinged lid and outside connecting switches for the various batteries is supported on rubber stoppers and placed inside a galvanized iron box not indicated on the diagram. This second shield may have been unnecessary and it would probably have been better to have improved the mechanical insulation of the tubes and placed each of them in a separate steel compartment with sufficient room to make the wiring straighter and simpler.

The input wire was protected by a grounded lead conduit with the exception of a short wire connecting it to the zinc electrode and of a double throw switch inserted for an alternative direct connection to the galvanometer. The amplifier was located on the opposite side of the room from the galvanometer, a distance of 14 feet, to protect it from the currents of the lantern, galvanometer magnet and camera motor. The camera motor and attachments were shielded with sheet-metal as was also the tuning fork, except for a small aperture for the reed attached to one of the prongs. The output wires were carried unshielded across the room to the galvanometer. When using the transformer the protecting resistances in the Leeds and Northrup box, designed by Williams for use with the string galvanometer, afforded sufficient protection. When the output condenser circuit was used a shunt switch S_8 allowed the condenser to be charged before connection was made to the galvanometer box through switch S_7. When connection is made to the string the added circuit must be charged through the string. It was, therefore, found advisable to connect an Ayrton

universal shunt across the string, as this affords a better protection against unforeseen potentials in the output, since it is necessary to start with a 0.0001 part of the output through the string and proceed through the fractions of 0.001, 0.01, 0.1 to the total output current. The leak of the condensers was so small that with a tense string compensation was unnecessary. With a loose string the leak was conveniently compensated by employing a second galvanometer box across the output terminals of the first.

It is of course necessary to have all the input connections and amplifier switches in position before starting to throw in the galvanometer. When using the condenser circuit disturbing the electrodes or touching the input will usually break the string if the galvanometer is connected. With the transformer circuit precautions of this sort are not so necessary.

All amplifiers, no matter how carefully designed have a tendency to sing, that is, to produce sustained oscillations of one or more frequencies. Most of our time has been taken up in minimizing this effect. Although we have been quite successful in this respect we are not yet certain just what factors enter into the production of a quiet system. It is necessary to have good electrical, mechanical and sound shielding of the apparatus. The wiring must be simple and avoid as much as possible inductance between the circuits of the different tubes. The ground circuit must be heavy, the leads from it must not be too widely distributed and the galvanometer ground should also come to the same common point. We feel that our electrical and sound shielding are adequate but that our mechanical shielding needs to be improved. The tubes themselves tend to sing less after they have been continuously in use for a long period of time.

The apparatus was tested for amplification. By this is meant the ratio of the deflection of the string of the galvanometer with the amplifier to that occurring without it. The magnitude of the effective amplification is a function of the resistance of the preparation. This is due to the fact that the deflection of the string depends upon the current going through it and, therefore, for a given potential is small when the resistance in the preparation is large; while on the other hand the vacuum tube tends to act as an electrometer. This follows because the current released by the tube is proportional to the alternating current voltage drop across the grid resistance. If e_n is the voltage developed by a nerve of resistance r_n, the voltage drop e_g across the 2 million ohm grid resistance is

$$e_g = e_n \frac{2{,}000{,}000}{r_n + 2{,}000{,}000}$$

The resistance of the nerve being small compared with 2 million ohms, changes in the resistance of the nerve produce but small changes in the voltage across the grid resistance.

When the deflection of a 3820 ohm string produced by 0.84 m. v. obtained from a potentiometer was compared with the deflection obtained when the same potential was impressed upon the grid of a one tube amplifier, the amplification was found to be only 8.7 per cent. When however 70,000 ohms, a resistance found in two of our nerve preparations, were added in the connection to the string or to the grid, the effective amplification became 27 times for one tube and 559 times for two tubes, an advantage of two tubes over one tube of 20.7 times.

Low operating efficiency, more apparent in the first instance, is due to power loss in the output and input circuits. The power developed in a string of a resistance r_o by a voltage e_g impressed upon the grid of a tube having a plate resistance r_p and an amplification constant μ is

$$\frac{\mu^2 e_g^2 r_o}{(r_p + Z_o)^2}$$ (Van der Bijl art. 63)

Z_o (defined below) can in the present argument be considered approximately equal to r_o. As the value of r_o is small compared with r_p the development of power in the string falls considerably below a maximum. When a source of potential e_n of internal resistance r_n is connected directly to the string the power developed in the string is

$$\frac{e_n^2 r_o}{(r_n + r_o)^2}$$

We have seen that e_n is nearly equal to e_g and therefore if r_n is small compared with r_o, as is the case when leading directly from the potentiometer, this expression may be as large as or larger than the one above. The tube acts as a generator of potential μe_g with a high internal resistance r_p. It operates into the galvanometer string which has a relatively low resistance r_o. For the power expended in the string to be a maximum r_o should equal r_p. Increased output efficiency could be obtained by matching the string impedance to the tube impedance through a transformer. Similarly matching the resistance of the source of potential to the grid resistance through an input transformer would step up the input voltage and increase efficiency.

It is usually for other reasons undesirable to use transformers. It is better to gain amplification by stepping up the input voltage e_n with additional tubes and it is by this means that the desired effective amplification from a tissue of any resistance may be obtained. The condenser output connection has been found to be generally more practicable than the transformer connection. Curves obtained with the latter must be integrated once and if there is also an input transformer twice, in order to obtain the form of the input wave. The transformer connection is nevertheless of value under some circumstances. It can be made to amplify more efficiently and it is as free from noise.

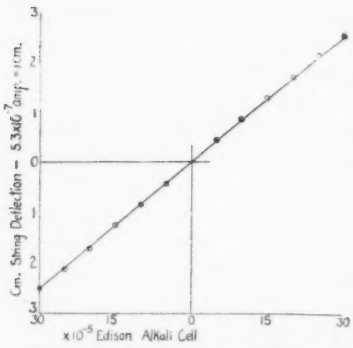

Fig 3. The points are a plot of the string deflection against known grid potentials when using two tubes and a 14 microfarad output condenser.

The conditions for distortionless amplification are that the impressed grid potential shall not be large enough to cause the grid to absorb current, that the dynamic characteristic of the amplifier shall be linear and finally that the amplification shall be independent of the frequency. The input potentials with which we are dealing are so small that the first condition does not enter into consideration. The second condition makes it necessary that the deflection of the string shall be a linear function of the input potential, as far as the increasing tensions of the string, on deflection, allow this to be true, and that the angle of external impedance shall be nearly zero. As a two tube condenser circuit was found to be the most useful we have plotted the deflections produced by such a system against the range of input potentials that will cause deflections of a tense string which do not exceed the limits of 6 cm. paper. In this range, which includes most of our working range, the amplification is practically a constant as the points all come very close to a straight line (fig. 3).

The impedance of a circuit is determined by its resistance, inductance and capacity and is the denominator of the expression

$$I = \frac{E}{\sqrt{R^2 + \left(2\pi fL - \frac{1}{2\pi fC}\right)^2}}$$

where I is the current in amperes, E is the potential in volts, R is the resistance in ohms, f is the frequency, L is the inductance in henries and C is the capacity in farads. The expression in the parenthesis is known as the reactance. The angle of impedance is the arc tangent of the reactance divided by the resistance.

The inductances of the amplifier circuit are all very small and may be neglected. The important reactances occur in the output and coupling condensers. The external portion of the alternating current circuit of the last tube is essentially the 14 microfarad condenser and the 3820 ohm string. The external portion of the circuit of the first

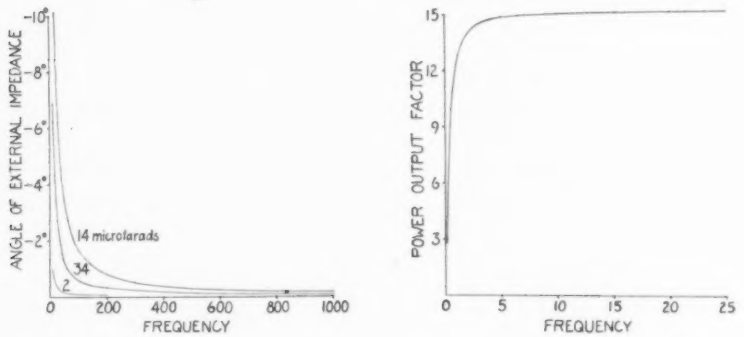

Fig. 4. *a:* The curves are the angles of impedance $\frac{xo}{ro}$, for the intertube circuit consisting of a two microfarad condenser and a 500,000 ohm resistance and for output circuits consisting of a 14 or a 34 microfarad condenser and a 3820 ohm string.

b: The curve is a plot of that fraction of the expression for power expended in the string which varies with the frequency of the impressed grid potential.

tube consists of a 2 microfarad condenser and a 500,000 ohm resistance. The angles of external impedance of these two circuits are plotted in figure 4 a. Both angles are quite small, especially for frequencies above one hundred. The total angle of external impedance is therefore small and the dynamic characteristic of the amplifier is substantially linear.

The third condition is satisfied if the impedances of the circuit do not vary sufficiently with the frequency of the input potential to distort the power amplification. The reactance of the coupling condenser is so small that it is only necessary to consider the effect of the condenser in the output circuit. In order to obtain a simple expression for the

power developed in the output circuit the last tube may be considered as being a generator of an alternating potential E, having a high internal resistance r_p. Omitting the small shunting effect of the 200,000 ohm resistance the impedance of the external circuit is

$$Z_o = \sqrt{r_o^2 + \left(\frac{1}{2\pi f C}\right)^2}$$

where r_o is the resistance of the string and C is the capacity of the output condenser. The current in the circuit is

$$\frac{E}{r_p + Z_o}$$

and the potential drop across the string is

$$\frac{E r_o}{r_p + Z_o}$$

The power expended in the string is the product of these two and equals

$$\frac{E^2 r_o}{(r_p + Z_o)^2}$$

The denominator only of this expression contains the factor which varies with the frequency and therefore the value of the expression

$$\frac{1}{(r_p + Z_o)^2}$$

for different frequencies is plotted in figure 4b. The amplification reaches 99 per cent of its maximum as soon as the frequency reaches seven per second and is thus practically independent of the frequency for frequencies above this value. The amplifier thus satisfies all the conditions for essentially distortionless transmission for frequencies above one hundred.

It is necessary however to consider the transient phenomena which occur in the circuit. If a potential $e = E\cos(\omega t - \theta_o)$ be impressed upon a circuit containing resistance, inductance and capacity in series, the expression for the current, i, in the circuit consists of a permanent and a transient term, namely, $i = \frac{E}{Z_o}\cos(\omega t - \theta_o - \gamma)$ + an exponential

term, (vid. Steinmetz (16)), where $\omega = 2\pi f$, t is the time, θ_o is the phase angle determining the instantaneous value of the impressed potential at the start, and γ is the impedance angle. The exponential or transient term depends not only upon the constants of the circuit but upon the nature of the impressed electromotive force and its phase at the moment of starting. In practice under our conditions the instantaneous starting potential is always zero, $\theta_o = 90°$. For the constants of our circuit and an alternating current of a frequency of one hundred per second starting when the instantaneous potential is zero, the transient term is a logarithmic curve, small in comparison with the maximum amplitude of the permanent term. During the period that the transient term is in operation the current records will oscillate about this curve as the line of zero potential. In case the impressed oscillatory potential is unidirectional, considerable energy is stored in the capacity before a permanent condition is reached and the transient term is a logarithmic curve whose initial value, as we shall see later, is large. Its slope is gradual and therefore only the low frequency harmonics are appreciably distorted during the period of several seconds in which the term is effective.

The current described by the permanent term, $\frac{E}{Z_o}\cos(\omega t - \theta_o - \gamma)$ has only a phase distortion which depends upon the values of f involved in the analysis of the impressed potential function. If the values of γ are small there will be but little phase distortion. Assuming that the negative variation in nerve lasts 5 thousandths of a second, the curve of potential can be analyzed into a fundamental of a frequency of one hundred per second and harmonics. The phase deviations of the fundamental and the ninth harmonic are respectively only 1.7° and 0.17° with a 14 microfarad condenser and 0.56° and 0.08° with a 34 microfarad condenser. The phase differences between the fundamental and the ninth harmonic are therefore 1.53° and 0.48° in the two cases.

There is a graphic way of looking at the distortion by the condenser circuit. When the grid potential is changed the tube resistance rises or falls and the potential of the condenser terminal must correspondingly rise or fall. A current will accordingly flow through the galva nometer between the condenser plates. The condenser reaches its equilibrium potential slowly if it has a large capacity to be charged through a high resistance. The charging (or discharging) current rapidly reaches its maximum under Ohm's law, then falls away logarithmically

as the condenser is charged (fig. 5). The falling off of the current in 0.01 second can not be measured with any accuracy but as the rate of change is logarithmic, it can be calculated for any point from any other two points. Such a calculation shows that the falling off is about 0.84 per cent in 0.01 second and not sufficient to produce an appreciable distortion in currents of short duration. In fact, changes as slow as the currents obtained by Einthoven from the vagus (17) are readily recorded, though with relatively more distortion. Figure 6 is included to show the form of the amplified electrocardiogram and its freedom from other vibrations.

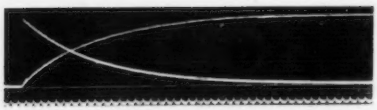

Fig. 5

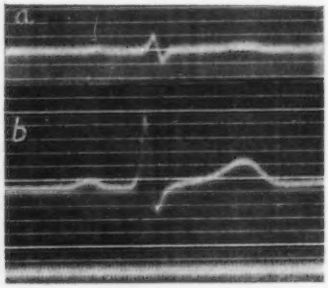

Fig. 6

Fig. 5. The deflection produced by a constant potential of 20 millivolts in a 3030 ohm string when in series with a 20 microfarad condenser and a 77,000 ohm resistance. The added line indicates the rise in potential of the condenser plate as the condenser fills. Time, $\frac{1}{5}$ second.

Fig. 6. Electrocardiograms taken from the right and left forearms by means of non-polarizable electrodes of the $ZnSO_4$ type having an area of contact of about 1 sq. cm. Time, $\frac{1}{10}$ second.

a, Connections made directly to the galvanometer.

b, Connections made to the galvanometer through a one tube amplifier. The conditions are otherwise the same as in *a*.

EXPERIMENTAL APPLICATION OF THE AMPLIFIER

The recording of the action current of the right phrenic of the dog was first tried with *amplification by one tube.* A typical result is shown in figure 7. The unamplified action current, *a*, may be compared with the amplified, *b*. The latter is selected from a series in which the string was progressively loosened to obtain the maximum oscillation, and presents a picture which requires some explanation.

The demarcation current in the nerve acts like any other battery producing a grid bias and contributes its part to the determination of the constant potential of the output condenser. The action currents produce deflections of the string downward. In addition to the individual deflections there is a sustained downward deflection of the string. This sustained deflection increases during the first portion of the inspiratory period, but the increase stops before the end of the period and very close to the end decreases rapidly, the string undergoing a rapid deflection upward to a point well beyond its equilibrium position.

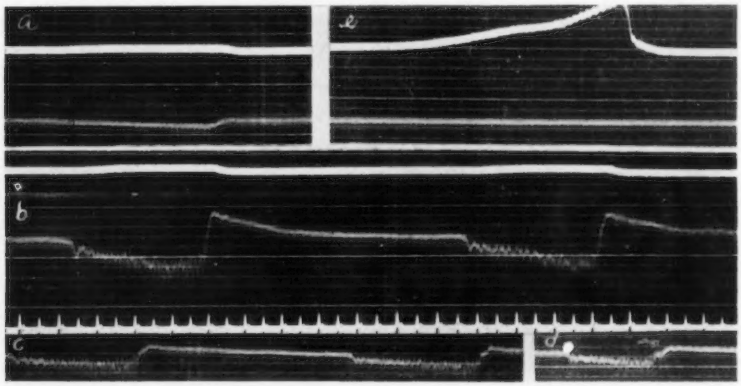

Fig. 7. Action currents from the right phrenic of the dog. Leads from the side and cut end of the nerve.

a: No amplification. Time same as in *b*, $\frac{1}{5}$ second. Upper line pneumograph, inspiration upward.

b: Amplification with one tube. Proximal electrode on the nerve connected to the grid; when it is negative the deflection is downward.

c, d: Same as *b* with string at greater tensions. The pneumograph and time tracings have been cut off.

e: Same as *b* except that nerve has been killed proximally to the proximal electrode. Time in *c*, *d*, *e* the same as in *b*.

This picture may be explained as the sum of the effects of the galvanometer, the increasing size of the individual nerve current waves and the condenser. It may be reproduced experimentally by recording a rapidly interrupted current from a potentiometer under similar conditions.

If unidirectional and equal changes in potential are impressed upon a tense string at intervals of the order of 1 one-hundredth of a second

the string will approximately follow the potentials so impressed and will oscillate between the zero axis and the maximum deflection about a mean position above the axis. This mean position is determined by the mean value of the potential function and is given by the definite integral of the potential in the time interval divided by that interval. If, on the other hand, the impressed changes are increasing in magnitude the line about which the string oscillates is no longer horizontal but is inclined from the axis. If under these conditions the string be loosened so that it no longer follows completely the rapid fluctuations in potential it will still oscillate about a mean position which is the mean as understood above. The distance of this mean from the axis will be greater because of the greater sensitivity of the string. The size of the individual oscillations, while it may be greater than obtained with a tense string, becomes a relatively smaller fraction of the deflection produced by a constant current of the same potential and therefore a smaller fraction of the curve of true potential.

In *a* and *b* of figure 7 the increasing distance from the zero axis of the line about which the string is oscillating is the result of an increase in size of the individual waves. In the latter part of an inspiration, in the record of the amplified current, it can be seen that in spite of the fact that the size of the individual waves is increasing the string begins to shift slowly upward. At this point the effect of the condenser, no longer masked by the increasing downward deflection, has become apparent.

The form of a complete condenser charging current is shown in figure 5. The quantity of current which has entered the condenser is measured by the area under the curve. On removal of the potential from the condenser terminals a curve of the same shape and size is obtained but it is in the opposite direction. If the charge of the condenser is not complete it is equal to the area under the portion of the curve which is completed and the initial current dissipating this charge is determined by the potential which the condenser has acquired and is measured by the difference between the initial and final charging currents. The area between the discharging current and the axis is equal to the area beneath the charging current. The time for complete discharge, being a matter of several seconds, is long compared with discharge intervals of less than 1 one-hundredth of a second with which we are dealing. The condenser thus receives with each wave a greater charge than it loses in the interval. The condenser accumulates a charge and a potential which results in interval discharge currents of increasing

magnitude. Equilibrium is established when the loss of charge in the interval becomes equal to the charge gained. The effect of the condenser is therefore to lower the line about which the string is understood to be oscillating until the line coincides with the zero axis.

In figure 7, *b*, the negative potential produces a downward deflection and the accumulating charge of the condenser an upward shift which is overbalanced by the increasing size of the waves during the first part of the inspiratory period. At the end of the period the rapid decrease in size of the waves results in a rather abrupt upward shift of the string due in part to a decrease in the mean about which the galvanometer string is oscillating and in part to an increase in the discharging phase of the condenser. On the cessation of the waves the string moves to a position above the zero axis commensurate with the potential which the condenser has accumulated during the interval. The discharging current has the usual logarithmic form.

The true potential waves of negative variation would be represented by an oscillation between a logarithmic curve connecting the initial zero of the string and the point of maximum discharge current and another curve determined by this curve and the line of mean oscillation.

When *amplification with two tubes* was tried it was found to be far superior to that obtained with one. Such a result is recorded in figure 8. This record was made with a string tightened to the border of periodicity. The tight string follows the individual waves and therefore the bases of the waves return to a line indicating zero potential. The progressive movement of the bases of the waves upward due to an accumulating charge on the condenser can now be seen without the complicating effect of the slow galvanometer string. Destruction of physiological continuity by heating or crushing a segment proximal to the proximal electrode caused all signs of action currents during inspiration to disappear.

A direct comparison was now made of the relative values of one and two tubes. The leads were, in this case both from the side of the nerve, but the connection at the diaphragm had been severed so that the nerve could be looped through the electrodes in the manner previously described. With the string tightened to the border of periodicity the sensibility was still such when two tubes were used that the oscillations exceeded the limits of the 6 cm. paper (fig. 9, *c*). A small amount of "noise" is visible in the respiratory pause but is insufficient to seriously interfere with the record.

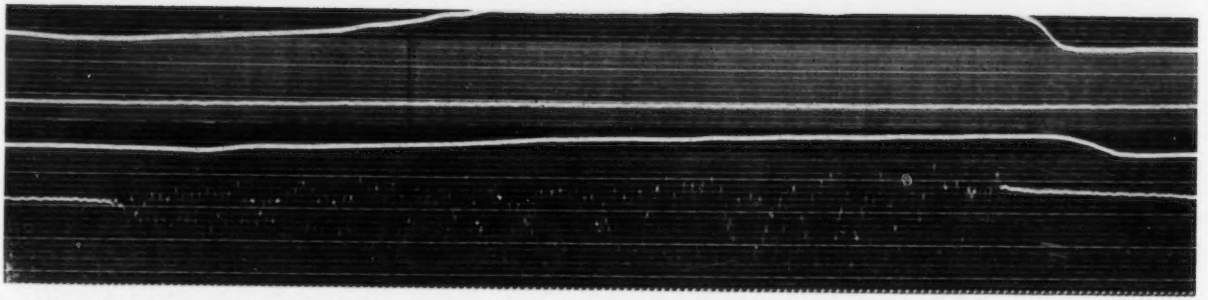

Fig. 8. The action currents of the left phrenic of the dog obtained with the aid of two tubes and an output condenser of 34 microfarads capacity. The distal electrode on the nerve is connected to the grid of the first tube. Negativity of the proximal electrode is indicated by a deflection downward. Time $\frac{1}{50}$ second, the same in both records. Calibration of the string 5.5×10^{-7} amperes = 1 cm. Upper line pneumograph, inspiration upward. The upper record is the same as the lower except that the nerve had been killed proximally to the electrodes.

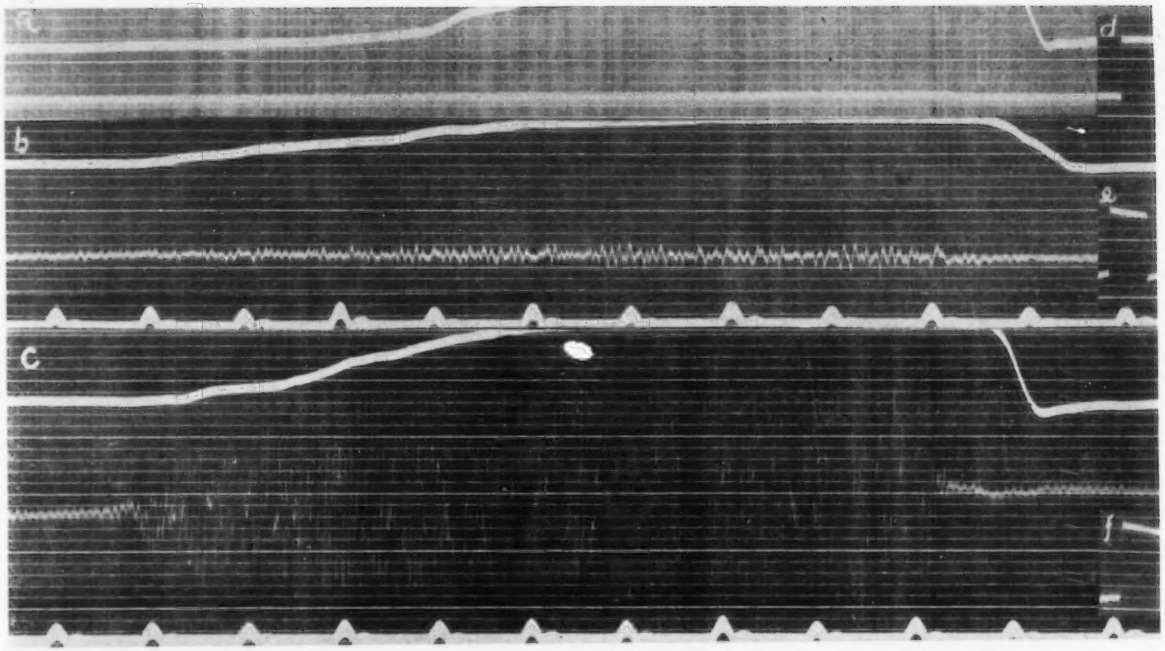

Fig. 9. Action current records from the right phrenic of a dog taken in as rapid succession as possible.

a: Unamplified. The leads are from the side of the nerve to the cut end; 9.3×10^{-8} amperes = 1 cm. The line above the string shadow in each record is the pneumograph shadow, inspiration is upward. The time record has been cut off, it is the same as in *b* and *c*, in $\frac{1}{5}$ second.

b: Amplification with one tube; 9.3×10^{-8} amperes = 1 cm. The leads are both from the side of the nerve. Resistance of nerve plus electrodes 110,000 ohms. Negativity of the proximal electrode produces a deflection downward.

c: Amplification with two tubes; 5.2×10^{-7} amperes = 1 cm. Leads as in *b*. Negativity of the proximal electrode produces a deflection upward.

d, e, f: Data for calculation of the amplification. String at a constant tension; 5.5×10^{-7} amperes = 1 cm. String resistance 3820 ohms.

d: No amplification; 84 millivolts across 110,000 ohms and the string.

e: One tube; 2.52 millivolts with 110,000 ohms in series in the grid circuit.

f: Two tubes; 0.168 millivolt with 110,000 ohms in series in the grid circuit.

This record may now be compared with *b* in figure 9, which was made from the same leads using one tube. The string was now looser, having been let out empirically to obtain the maximum oscillation. It is readily seen that under these conditions the distinctness of the individual oscillations has been sacrificed in the interest of amplitude. As the potential changes when this lead is used are not unidirectional the slow changes in the position of the string which have been described above are negligible. Without amplification no visible oscillation appeared in the string when it was used at a tension as great as that employed in recording the output of two tubes, therefore the string was not photographed. Figure 9, *a*, shows an unamplified record from the nerve when the distal lead was changed from the side to the cut end and the tension was changed to an optimum. As the leads from the side of the nerve were well separated the resistance of the nerve was large, 110,000 ohms. The effective amplification was therefore also large, the factor being 36.5 for one tube and 624 for two tubes.

OBSERVATIONS ON THE ACTION CURRENTS OF THE PHRENIC NERVE

The waves of negativity appear in the record at the beginning of an inspiration. They increase gradually in size during the early part of the cycle and attain a maximum which they hold to close to the end of the cycle when they terminate much less gradually, sometimes quite abruptly. An envelope of the crests would therefore have a shape roughly corresponding to the pneumograph shadow. This indicates that the gradual even contraction of the diaphragm is occasioned by the calling into activity of more and more nerve fibers as inspiration deepens. On the other hand the more abrupt falling off of the phrenic action current corresponds with the well-known fact that the expiratory limb of the respiratory tracing is much steeper, expiration occurring passively as the innervation to the muscle is withdrawn. In none of our records have we seen any evidence of innervation of the diaphragm during the intervals between inspirations.

While variation of the average size of the waves occurs in different parts of the respiratory cycle, the average rate seems to be constant throughout. In some records the last five or six waves appear at intervals smaller than the average but this is not a constant occurrence. The action current has the appearance that would be produced by discharges from the cord closely resembling volleys. The intervals between the volleys, while they show considerable regularity, are by

no means constant even when the waves are most smooth and regular. On the other hand the variation in the rate at which the waves appear is not as great as their difference in amplitude.

The waves are definite enough throughout an inspiration so that their total number can be determined with only a small percentage of error. They were counted in a number of experiments and the results are listed in the following table.

TABLE I

	NUMBER OF WAVES IN ONE INSPIRATION	DURATION OF INSPIRATION	RATE PER SECOND
		seconds	
Dog A	138	1.59	87
	115	1.29	89
	120	1.30	92
	108	1.24	87
Dog B Consecutive inspirations	176	1.90	93
	184	1.89	97
	178	1.88	95
	175	1.80	97
	159	1.75	91
Dog C	145	1.43	101
	144	1.39	103
	149	1.40	106
	113	1.16	97
	126	1.21	104
Dog D	78	1.105	72
	90	1.21	74
	80	1.095	73

No generalization as to rate is justifiable from these data. They only hold for the conditions of the experiment. The temperature of the animals was not controlled. While precautions were taken to prevent cooling, the temperatures of the animals were undoubtedly several degrees centigrade subnormal. The rates recorded in the different experiments vary from 87 to 106 per second. In one animal in poor condition the rates counted were 71 to 74. The normal innervation rate would probably be higher, not lower.

In a number of records made on rapidly moving paper the intervals between the consecutive waves throughout the period of activity were carefully measured in the search for some evidence of system in the

irregularity. For instance in one inspiration of 138 waves, the average separation was 10.95 σ; the variation was from 8.8 σ to 17.9 σ. Excluding 22 of the extreme intervals, the variation from the average was from minus 13 per cent to plus 19.6 per cent. In the most regular record obtained, where the rate of discharge was slower (71 per second), in 61 consecutive volleys of a series of 78, 77 per cent were within 10 per cent of the average. But no evidence of any recurrence in the irregularity was visible.

In addition to difference in size and distance apart, there is also a variation in the shape of the waves. To account for the latter the possibility of differences in phase and of differences in period in the different fibers must be considered. In referring to a difference in phase it is intended to designate a condition in which the nerve fibers are all alike, the velocity of propagation of the nerve impulse and therefore the period during which the electrode is affected by the negative variation being the same in all, but in which, due to the fact that the disturbance is not started in all the fibers at the same instant, it does not reach a given level in the nerve in all fibers simultaneously. It is clear, however, that there cannot be many different phases or the wavelets would either be very large in number or they would fuse producing a waveless record. Furthermore the fibers cannot be mutually far out of phase or it would be unlikely that groups of large waves free from superimposed waves would be obtained. At times all the fibers are very close to being in phase because the individual action currents have the well-known appearance of the action current obtained in nerve or muscle from a single induction shock. This can be seen in figure 8, in which it should be borne in mind that negativity of the proximal electrode produces a downward deflection. To bring this fact out more clearly, a record, taken on paper moving 25 cm. per second and from a nerve in which the rate of discharge was slow (71 per second), was redrawn. The points used for plotting were taken with the aid of a binocular magnifier. In the graph, the ordinate (deflection of the string) was unaltered in size, but the abscissa (time) was multiplied by 5. This gives the appearance which the record would have if the photographic paper had been run 5 times as fast. The appearance of both a monophasic and a diphasic lead from the same nerve is thus shown in figure 10.

If the impulses are discharged into the nerve at regular intervals one would expect that if they are merely out of phase there would be some tendency of the curves to repeat themselves. This would also be true if the component waves have a different period. Differences

in period could arise from the fact that the duration of the negativity under the electrode is not the same in all elements of the nerve, a condition arising from different velocities of conduction as a result either of physiological differences in the constituent fibers or of variation in exposure to injury. In the latter case there would be a tendency toward beat production, and of this the records show only the faintest tendency. We must, therefore, consider that the intervals at which the spinal cord discharges impulses into the nerve are within limits irregularly variable, that the number of fibers involved in the individual volleys varies and that the disturbance is started at times nearly simultaneously in all the fibers; at other times there is a slight difference in phase which may become so large that accessory waves may appear on the main wave.

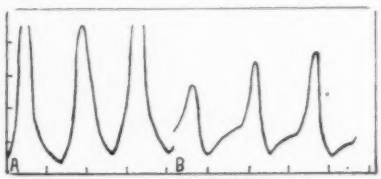

Fig. 10. Action currents of the phrenic nerve plotted from a record. *A*, monophasic; *B*, diphasic, from the same nerve. Ordinates, same as in record, 1 division = 1 cm. Abscissae multiplied by 5, 1 division = $\frac{1}{100}$ second.

Considerable interest lies in an estimation of the rate of discharge through an individual fiber. The difficulty in determining this rate lies in the waves that may be considered either as a portion of one volley somewhat out of phase or of another volley involving perhaps a small number of fibers. The error in counting on account of such waves is fortunately not as large as might be anticipated. The fair accuracy of the counts recorded in table 1 appears in the fact that for a wave in the nerve a corresponding one can be obtained simultaneously in the muscle. This was done with two galvanometers arranged tandem on the optical bench, the image of the string of the first galvanometer being focused by the second in the same plane as the image of its own string.

One galvanometer was connected to the anterior part of the right side of the diaphragm, the two electrodes lying on a radius from the motor point and in such a way that a diphasic action current was obtained with the first phase upward when the proximal portion of the muscle was negative. Two leads were made from the side of the right phrenic to the amplifier. The proximal electrode was connected to the grid of the first tube. Two tubes were used, but in spite of as complete isolation of the nerve as possible only one-tenth of the output could be recorded on account of extraneous currents, especially the

electrocardiogram. An idea of the effect of the latter may be obtained from the fact that in a record in which the whole output was recorded the "P" wave was 4 cm. high and the "R" wave was 7.8 cm.

Figure 11 is a record of the action currents of the right phrenic nerve and the right side of the diaphragm, taken simultaneously during one inspiration. Lines have been drawn from each wave on the phrenic nerve record to the corresponding one on the diaphragm record. The slope of the lines is apparent; the crest of a wave in the muscle action current comes about 9 σ after the crest of the corresponding wave in the nerve action current. This measurement does not allow for the

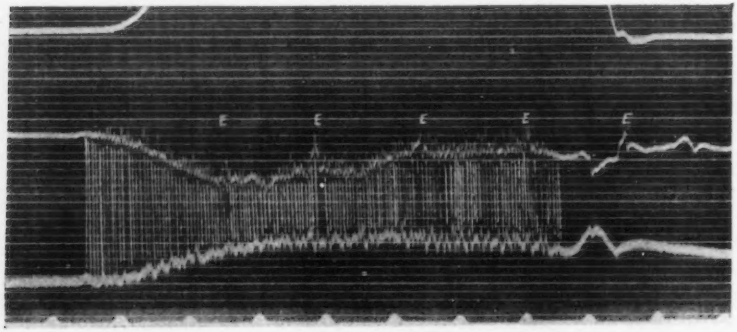

Fig. 11. Upper line: pneumograph, inspiration upward. Upper galvanometer tracing: leads from the side of the right phrenic nerve. Two tubes; $\frac{1}{10}$ of the output is recorded; 5.2×10^{-7} amperes = 1 cm. Deflection upward when proximal electrode is negative. Points marked *E*, electrocardiogram.

Lower galvanometer tracing: Action currents of the right side of the diaphragm recorded simultaneously with the nerve action currents. Negativity of the proximal electrode produces a deflection upward. Time in $\frac{1}{5}$ second.

phase lag of the loose string, recording the muscle action current, behind the tense string recording the nerve. Throughout the whole record corresponding waves can be identified. Moreover, there is a close correspondence in the size of the waves in the two records. The action current waves of the muscle are unevenly spaced but in every case it will be found that in the nerve record, preceding them by 9 σ, there will be waves with corresponding intervals. This is more clearly brought out in faster records.

An interesting observation is the symmetry of the action current records on the two sides. Records were made from the right phrenic

nerve, leading from the cut distal end and the side. This allowed the recording of the whole output from two tubes. Simultaneous with this was recorded the action currents of the opposite side of the diaphragm. The uneven spacing of the waves appears in both records but the intervals are the same on the two sides. While more data would be desirable on this point, the evidence indicates that the intervals at which the impulses are discharged from the cord into the nerves of the two sides is controlled from the same common point.

In comparing the nerve action current with the muscle action current recorded with a loose string it is often apparent that a very small wave in the muscle record, appearing sometimes as a notch on the side of a larger wave, has as its counterpart in the nerve record a definite wave. It must, therefore, be counted as a separate volley and not as a portion of some other volley out of phase, as is sometimes done. Piper's conclusions have been criticised in that, while the individual action currents in the muscle may be determined by corresponding disturbances in the nerve, the rate at which they appear is not fifty. We are not prepared to enter into this discussion at length. The rate of discharge into the phrenic nerve, even under the adverse experimental conditions, was twice this. The rate of the waves obtained from the forearm must depend on the technique employed and the basis on which they are counted. In our experience, when leads were made with small electrodes of one square centimeter surface from two points distal to the muscle equator, and recorded, after amplification, with a tense string, the rate was definitely one hundred or more.

SUMMARY

An apparatus is described by which physiological action currents as they appear in the phrenic nerve may be satisfactorily recorded.

This is done by a two stage three electrode vacuum tube cascade, resistance coupled, and connected to a string galvanometer through a large condenser.

The following points are brought out by the action current records:

1. Inspiration is brought about through discharge into the diaphragm of 78 to 178 volleys of impulses at the rate of from 71 to 106 per second, under varying conditions of the experiments.

2. The waves vary in size, suggesting variation in the number of nerve fibers involved. The average size of the waves is larger near the end of inspiration.

3. At slow rates of discharge the typical form of an artificially produced action current is apparent. Variations in the form occur and are probably caused by the fact that the discharges by the cord into the different fibers are slightly out of phase.

4. The intervals between the volleys are irregular.

5. Simultaneous records taken from the phrenic nerve and diaphragm show that every wave in the muscle is produced by a corresponding one in the nerve. The waves correspond in size and in the intervals of separation in the two records.

6. The similarity in the spacing and size of the volleys on the left and right sides suggests that the discharges from the cord into the two phrenic nerves are controlled from some common point.

We wish to thank Mr. R. E. Bitner and Mr. I. B. Crandall for helpful advice.

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THE OUTPUT OF THE HEART IN DOGS

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About twenty-five years ago (1) I described a method of measuring the amount of blood passing through the lungs and of following changes in that amount A considerable number of measurements on dogs anesthetised with morphine and ether, or with morphine and ACE mixture were published. The principle of the method is that a solution of a substance which can be easily recognized and quantitatively estimated in the blood is permitted to flow for a definite time at an approximately uniform rate into the heart. The injected substance mingles with the blood and passes out with it into the circulation. At a convenient point of the vascular system (usually from a branch of the femoral artery) a sample of blood is drawn off just before the injection and another during the passage of the substance; and the quantity of the solution which must be added to a given volume of the first sample, in order that it may contain as much of the injected substance as the second sample, is determined. This determination gives us the means of estimating the extent to which the injected solution has been mixed with blood in the heart, and thus, knowing the quantity of the solution which has run into the heart, we can calculate the output in the given time. I used a solution of sodium chloride for injection. The arrival of the mixture of blood and solution at the point of collection is easily detected by exposing a portion of the opposite artery at the same level as the collecting cannula, placing it on electrodes connected with a Wheatstone's bridge, and balancing the bridge till the sound in the telephone is a minimum. As soon as the blood mixture reaches the point of collection the balance of the bridge is upset, the sound increases more or less rapidly (according to the velocity of the blood and the quantity and strength of the salt solution injected) to a maximum, at which it remains steady till the greater part of the column of altered blood has passed, and then declines again more or less rapidly. When

the injection is made into the left ventricle, which is preferable for most purposes, but not indispensable with salt solution, the interval between the commencement of injection and the beginning of the sound is, of course, much shorter than when the solution is injected into the right heart. Also, the maximum is more rapidly attained and the sound disappears again more abruptly. The question whether the salt solution is uniformly mixed with the blood in the heart, when the injection is uniform and the inflow of blood into the heart steady, was examined and answered in the affirmative. Successive samples, collected while the blood mixture was passing and while the sound remained steady, had approximately the same conductivity.

In the majority of the observations the salt solution was allowed to run into the heart for so long a time (10, 12 or even occasionally 15 seconds, depending on the velocity of the blood stream and the size of the animal) that the *rate* of injection could be accurately estimated. The time for which a uniformly altered column of blood continues to pass the point of collection is, of course, increased with the time of injection. A sample collected during the time when the sound remains steady, or a considerable portion of that time, will obviously show the increase of conductivity which the salt solution entering the heart at the given rate is maintaining in all the blood passing through the heart. Thus, if m cc. of salt solution must be added to 1 cc. of the normal sample to make its conductivity equal to that of the sample collected during passage of the blood mixture, and Q cc. of solution in T seconds, i.e., $\frac{Q}{T}$ cc. of solution per second was injected into the heart, then $V = Q\frac{60}{mT}$, where V is the volume of blood passing through the heart in a minute (procedure I).

Besides reckoning the output from the maximum steady increase in concentration of the injected substance in the collected sample and the rate of injection, it can be estimated in another way, which was also employed by me. The blood is collected for the whole period during which the mixture is passing the collecting cannula, including the preliminary period when the concentration is increasing to the steady maximum and the final period when it is diminishing again. The amount of salt solution necessary to raise the conductivity of the normal sample to that of the sample collected in this way during passage of the mixture will clearly be less than that required for a sample collected only at the maximum concentration, and the greater the "tailing off"

of the column of blood, the smaller will be the increase in the specific conductivity of the whole sample. This does not affect the result, however, provided that instead of the rate of injection, we take the total quantity of solution injected and consider that this quantity has mingled with all the blood passing through the heart, not during the time of injection but during the time of collection of a complete sample of the blood mixture. That this assumption is correct, so long as the output of the heart remains steady, is easily seen if we consider that the first and last parts of the column represent mixtures in which a given mass of injected salt has been distributed over a larger quantity of blood coming to and leaving the heart than when the steady state has been reached. The greater amount of blood with which the salt solution has mingled, calculated from the smaller average increase of conductivity of the entire sample, will, however, be precisely balanced by the increased time over which this greater quantity of blood is being discharged. The same is true even if collection of blood is begun at the moment when the injection commences or at any time between that moment and the arrival of the first portion of the mixture at the point of collection. For the normal blood added to the sample will dilute it in proportion to the extra time of collection. Thus, if Q is the number of cubic centimeters of salt solution injected, t the time of collection of the sample of the blood mixture in seconds, and m the quantity of salt solution (expressed as a fraction of a cubic centimeter) which must be added to 1 cc. of the normal sample to make its conductivity equal to that of the blood mixture, then $V = Q\,\frac{60}{mt}$ where V is the number of cubic centimeters of blood passing through the heart per minute (procedure II).

Or, making the formula a little more general, $V = Q\frac{C}{c}\cdot\frac{60}{t}$ where V, Q and t have the same significance as before, while C represents the concentration (number of grams per cubic centimeter) of the injected substance in the solution, and c its concentration in the sample of blood mixture collected. Provided that the rate of collection is uniform, c will not depend upon the rate. For $c = \frac{vc_1t_1 + vc_2t_2 + vc_3t_3 + vc_4t_4}{v(t_1 + t_2 + t_3 + t_4)} = \frac{c_1t_1 + c_2t_2 + c_3t_3 + c_4t_4}{t}$, where v represents the number of cubic centimeters of blood collected per second, c_1 the average concentration of the sample collected in t_1 seconds while the concentration is rising to the maximum, c_2 the concentration of the portion collected in t_2 seconds while it is steady at the maximum, c_3 the average concentration of the portion collected in t_3 seconds while the concentration is declining to zero or to the minimum, and c_4 the concentration of any portion collected in t_4 seconds before or after the blood

mixture has passed the cannula. When a salt not present in the blood is used for injection c_4 is zero and the term c_4t_4 drops out without increasing the numerator. The time t_4, however, increases the denominator, and c is diminished by its inclusion, in the same measure as t (the sum $t_1 + t_2 + t_3 + t_4$) is increased. Precisely the same thing happens, if sodium chloride is injected, c, c_1, etc., then representing the average increase of concentration (or rather, as the method is applied by us, of conductivity) above that of the normal specimen of blood.

In some cases the outputs calculated in the two ways were compared in successive observations on the same animal. For example, in experiment XVI (table 3) the observation at 2:56 was made with collection of a sample with maximum concentration of the salt; 18.6 cc. of salt solution were injected in 7 seconds and collection made from 8 seconds to 15 seconds after the beginning of injection, while the sound was steady at the maximum. The output, calculated from the maximum concentration (procedure I) was 95 cc. per minute per gram of bodyweight. In the next observation, at 3:11, 10.6 cc. of salt solution were injected in 4 seconds, and collection made from 7 seconds to 15 seconds after the beginning of injection, embracing the whole period of passage of the mixture. The output calculated from the average concentration of the whole column (procedure II)was 95 cc. per kilogram of bodyweight. Of course, an exact agreement is only accidental in measuring a quantity like the cardiac output. The total time of injection should be shorter with procedure II than with procedure I, so that collection may be completed before a round of the circulation has been made by any appreciable part of the salt. Henriques (2) has discussed this point in his excellent paper.

In experiment XX (table 4) the observations at 10:46 and 11:04 were on samples collected during the whole time of passage of the blood mixture, the output being calculated by the formula $V = Q\frac{60}{mt}$. In the other observations in this experiment the sample was collected only while the sound was steady at the maximum, the output being calculated by the formula $V = Q\frac{67}{mT}$. The agreement is about as close as is often obtained in two measurements by either of the two procedures.

In 1913 Henriques (2) employed the method, using the second procedure (injection of a definite quantity of a detectable substance and collecting during its whole time of passage). Instead of sodium chloride he injected sodium sulphocyanide. He had no means of knowing when the substance reached the collecting cannula, and therefore employed a very long period of collection, beginning, indeed, as soon as the injection started. His results were obtained under

highly abnormal conditions, his object being to check the conclusion previously come to by Bohr and himself (3), that a substantial fraction of the total combustion in the body takes place in the lungs. For this it was necessary to know how great the coronary blood flow was, in comparison with the total output of the heart, in animals under the conditions of the original experiments. All the branches from the aortic arch (including the innominate and the left subclavian) were tied. The animals received morphine, curare and also leech extract to prevent clotting. Artificial respiration was of course employed. The blood pressure was low in most of the experiments (only 5 are given), and the output of the heart was correspondingly small. But there is no reason to doubt that for the conditions purposely imposed the output was determined with considerable accuracy. For comparison, the results of the 5 experiments, with 3 others (7, 8 and 9) from a second paper (4), are brought together in table 1. It will be seen that in nearly all the experiments there was a progressive decline in the output, accompanied by a fall of blood pressure. I have calculated the standard deviation (σ) and the probable error of the means, which of course is most satisfactory for a series of pretty uniformly distributed values like those in experiment 5 and for a larger number of separate determinations. In this experiment there is a very good agreement between successive determinations. It must be noted, however, a fact which is not always remembered by writers on the subject, that the cardiac output is not a constant like a refractive index or a melting point, but under ordinary conditions a variable quantity, not only in different individuals but in one and the same individual. By limiting voluntary movements, eliminating changes in respiration, cutting out the extrinsic innervation of the heart, or at least the cardio-inhibitory fibers, damping down the metabolic and other variations of the organism by deep anesthesia, a fairly close agreement in successive determinations of the cardiac output in one and the same animal may be procured.

The experiments of Barcroft and his collaborators (9) on unanesthetised goats show generally a very good agreement between separate measurements on the same animal at an interval of several hours or even days, provided that the animal remained quiet during the short time necessary for an observation. Anesthesia is, of course, no guarantee of uniformity in successive observations, made even at short intervals, if the depth of the anesthesia varies or the general condition of the animal (blood pressure, etc.) changes. And it is not difficult to see that under anesthesia the conditions essential for a relative uniformity might alter more rapidly than without anesthesia. However even under the best conditions, an agreement like that between duplicate determinations in a chemical analysis would appeal rather to a chemist than to a physiologist in relation to such a quantity.

I rather insist upon this point because in a recent paper Bock and Buchholtz (5), who have introduced my method for the study of the effect of drugs upon the output, seem to imagine that when they get two successive determinations as close, for instance, as 176 and 179 cc. per kgm. of bodyweight per minute, this is in itself a proof of good technique. Their technique is good, and in one point (the securing of a more uniform injection) they have improved on the original technique. In my opinion, however, they (and also Henriques) have lost something of value in not being able to know the actual time of arrival at the point of collection of the substance injected by them until after the experiment, so

that they have to guess at the proper time to collect their samples. This would be especially a drawback in studying the output under conditions where considerable changes were occurring in the velocity of the blood stream. Bock and Buchholtz employed the first of the two procedures discussed above, determination of the maximum concentration of the injected substance and the rate at which it is injected. They seem to consider that their experiments were done according to a somewhat different principle than mine. "Ferner richteten wir unsere Versuchsanordnung nach einem etwas anderen Princip ein als Stewart und Henriques." The fact is that Bock and Buchholtz used the principle of our first procedure exactly, while employing a different substance (sodium iodide) for injection, while Henriques employed our second procedure, but with sodium sulphocyanide. Both of these substances are stated to be harmless in the quantities employed, but they cannot possibly be superior to a weak sodium chloride solution in this respect. Both sulphocyanic acid and iodine can be estimated with considerable accuracy, but it is difficult to see how this can be done more accurately than the estimation of differences in the conductivity of blood, such measurements being among the most exact physical determinations. Much the greatest part of the increase of conductivity of the blood when salt solutions in the quantities used in these experiments are mixed with blood is due to the increased sodium chloride content of the plasma, a small part to the increased volume of plasma relatively to corpuscles (6). Both factors act in the same way whether the mixture is effected in the heart or in a test-tube. The determination of the amount of salt solution needed to equalize the conductivity of the normal specimen with that of the specimen collected during passage of the mixture gives, therefore, precisely the same information as the estimation of sulphocyanide or iodide when these salts are injected. It need scarcely be pointed out that the volume of liquid actually ejected by the left ventricle, in the period for which the output is determined, is the volume of blood which enters the ventricle *plus* the volume of the injected liquid. No correction is necessary for this, as it is the volume of blood mingling in the heart with the solution which is measured.

But it is less easy to take account of another factor when numerous injections of solution are made and numerous samples of blood withdrawn in successive observations over a short period of time, namely, the possible changes in the total volume of the circulating blood. Excluding exchange of liquid between the blood and tissues due to alteration of the composition of the blood by the injections (which, of course, cannot generally be completely excluded), no variation in the total blood volume will be caused throughout an experiment if the volume of blood withdrawn is kept equal to the volume of solution introduced. This was approximately the case in our experiments, as shown in table 2. However, with repeated injections the volume of the plasma will increase relatively to that of the corpuscles, unless the volume of liquid injected is small and the time between successive injections sufficiently long to permit the reëstablishment of equilibrium. The vicsosity of the blood may therefore be expected to diminish as the experiment proceeds, and in most of our experiments this was clearly seen. We have no definite, uncomplicated observations to show whether a diminished viscosity would of itself cause a greater minute volume.

Bock and Buchholtz have laid stress on the necessity of a uniform rate of injection (when the first procedure is used), while it need not be uniform with the

second. In a general way this is true, but when a sample is collected during the whole period of maximum change in the blood mixture or a great part of it, rapid variations may occur in the rate of injection without detriment so long as the average rate remains constant. Conversely, if the rate of injection is kept uniform, rather rapid variations in the quantity of blood coming to the left ventricle will produce no change in the sample collected according to the first procedure, so long as the average minute volume does not vary.

Henriques states that he was unable to obtain satisfactory results with injection of sodium sulphocyanide into the right side of the heart. It is probable that a portion of the sulphocyanide is retained in the lungs, which would cause the calculated output to be too large. Further, owing to the interposition of the pulmonary circulation the maximum concentration was too slowly reached, and the subsequent decline to the minimum was also slow. We have a far larger mass of data on the measurement of the pulmonary circulation time by the electrical conductivity method (7), with injection of sodium chloride solution. A good maximum is obtained, from which the output can be calculated, with injection of such quantities of salt solution as are permissible, although it is advisable to use larger quantities than those sufficient when injection is made into the left ventricle. As illustrated in experiment XXII (table 4), the results obtained by injection into the right heart are concordant with those found in the same animal with injection into the left heart. But here, of course, the telephone informs the observer of the time of occurrence of maximum and its duration. And if an insufficient amount of salt solution has been injected to give a sharp result, he can modify this at will, while the experiment is going on. This could not be done in Henriques' observations. There is no obvious reason why our first procedure (collection of the specimen at the maximum concentration) should not be applicable when the blood mixture has to traverse a capillary area, especially one with such a short and relatively constant circulation time as the lungs. To be sure, the period of increasing concentration before the maximum is reached will be somewhat lengthened, but it is difficult to see from the principle of the method how this can introduce an error so long as the steady maximum concentration lasts long enough for a good sample to be collected, which it certainly does with injection of sodium chloride solution.

Tigerstedt (14) appears to contend that because v. Kries (8) made experiments which confirmed the theoretical conclusion, that the mean velocity of a stream of water flowing through a straight capillary tube of certain dimensions is half the maximum velocity, owing to the uniform increase in velocity from the periphery to the axis, the circulation time through a capillary area, determined by noting the interval after injection of a salt at which the first portions of the salt arrive at a large vein draining the area, or in the case of the lungs at the carotid artery, must also be half of the mean circulation time. I am quite unable to understand how a given particle of salt solution, passing through a network of capillaries between corpuscles which are rubbing on the capillary walls, jostling each other and often temporarily blocking the capillary lumen, can remain always in the axial stream and always moving with the maximum velocity. It is much more probable that its velocity will vary considerably in its transit through the capillaries and will not differ much from the mean velocity of the whole of the blood. This conclusion finds confirmation in experiments performed on artificial capillaries (1).

TABLE 1

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	BLOOD PRESSURE
	kgm.		*grams*	
1	16	10:48	71	115
		10:59	70	115
2	17.2	10:48	62	55
		10:57	58	55
		11:02	49	40
		11:13	45	25
		11:20	40	17
Average			51 ± 2.4; (σ 8)	
3	21	10:48	46	55
		10:54	42	30
		11:01	35	23
Average			41 ± 1.7	
4	8.5	11:05	113	85
		11:11	94	80
		11:19	78	40
		11:23	70	35
		11:27	57	35
		11:31	58	30
Average			78 ± 5.5; (σ 20)	
5	9	1:44	68	60
		1:48	86	70
		1:54	85	80
		1:58	82	80
		2:02	78	100
		2:06	81	100
		2:11	85	110
		2:16	84	105
		2:21	76	110
Average			80 ± 1.2; (σ 5.5)	
7	21		29	
8	22	10:34	40	
		10:39	39	
		11:28	48	
		11:33	35	
Average			41 ± 1.6; (σ 4.7)	
9	20		44	75*
			37	

* Blood pressure was raised by injection of strophanthin before the measurement.

My experiments on the output of the heart were planned fully as much to investigate the changes which might occur in its magnitude, when the circulatory conditions were greatly altered, as to obtain results which might be employed for calculating a so-called "normal average" for the output, a quantity whose variability is one of its chief characteristics. For this reason a number of the experiments were carried on to the point where the animal had markedly deteriorated and the circulation was failing. Experiments XVI and XIX (table 3) are samples. In the latter part of experiment XVI the pulse rate

TABLE 2

DOG	BODY WEIGHT	NUMBER OF OBSERVATIONS	DURATION OF EXPERIMENT	BLOOD DRAWN	SOLUTION INJECTED
	kgm.		*minutes*	*cc.*	*cc.*
XII	15.25	6	60	113	162
XIII	27.89	10	130	300	308
XIV	32.26	9	90	340	344
XV	4.97	4	110	53	71
XVI	11.79	10	95	250	251
XVII	18.20	12	125	220	207
XVIII	9.89	7	60	179	174
XIX	12.82	7	70	237	158
XX	10.32	5	50	148	92
XXI	14.99	9	140	184	193
XXII	17.5	16*	120	386	394
XXIII	7.15	5	40	91	81
XXIV†	34.55	11	145	300	245

In all the experiments the number of blood samples collected was approximately double the number of observations of the minute volume.

* Of these observations 5 had to be rejected because of unsatisfactory injection into the left ventricle, chiefly owing to insufficient pressure. In these 5 observations 27 cc. of the 1.5 per cent NaCl were introduced and 103 cc. of blood collected.

† Injection into descending aorta.

increased rapidly, the heart became progressively weaker and the output was soon cut down to one-third of its initial value. In experiment XIX the same thing was seen. The pulse became continually feebler, especially from the 4th observation to the 7th (inclusive). Precisely the same thing is seen in the experiments of Henriques (expers. 2, 3 and 4, table 1). The relative decrease in the output in his observations could not be quite as great, simply because the initial output, owing to the experimental conditions, was abnormally low, and the blood pressure rapidly fell to an extremely small value.

The opposite effect of artificially induced hydremic plethora is illustrated in experiment VII (table 3). In the course of the experiment 125 cc. of 5 per cent sodium chloride solution were injected through a catheter passed into the right external jugular vein. *In vitro* this would have trebled the molecular concentration of the original quantity of plasma. The conductivity of the blood drawn off at the end was

TABLE 3

EXPERIMENT XVI. WEIGHT 11.79 KGM.		
Time	Minute volume per kilogram	Heart rate
	cc.	
1:57	318*	94
2:02	290*	99
2:09	233	96
2:17	174	120†
2:43	226	130
2:50	107	130
2:56	95	134
3:11	95	127
3:20	101	127
3:30	79	182
Average output (first 5 observations)	248	
Average output (last 5 observations)	95	

EXPERIMENT XIX. WEIGHT 12.82 KGM.		
Time	Minute volume per kilogram	Heart rate
	cc.	
1:08	346	98
1:23	297	110
1:32	191	106
1:46	181	128
1:54	140	130
2:01	121	156
2:17	102	170

EXPERIMENT VII. WEIGHT 6.48 KGM.	
Minute volume per kilogram.	Heart rate
cc.	
157	83
189	
226	98
295	97

* These two measurements are too high, as collection of the sample was begun simultaneously with the arrival of the first portion of the blood mixture before the sound had reached its maximum, and finished while it was still at the maximum.

† At this point the pulse rate increased abruptly and the heart beat became progressively weaker.

In experiment VII morphine and ether, in experiments XVI and XIX morphine and ACE mixture were the anesthetics.

twice as great as that of a sample taken at the beginning of the experiment. The progressive increase in the output is evident, the final measurement, while the animal was still in good condition, being nearly double the initial output.

The rest of my observations are summarized in table 4, which contains about 100 separate determinations on 14 dogs. There is reason

TABLE 4

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	HEART RATE
	kgm.		*cc.*	
XIV	32.26			68
			199[1]	84
			150	88
		4:24	146	92
		4:30	141	98
		4:45	170	98
		4:55	170	98
		5:05	159	146
		5:15	139	148
		5:25	169	150
Average			160 ± 4; (σ 11)	
Average (last 8 observations)			155 ± 3; (σ 12)	
XIII	27.89	3:30	110	68
		3:52	94	68
		4:09	146	70
		4:14	113	70
		4:26	115	70
		4:36	106	105[2]
		5:01	109	145
		5:11	79	158
		5:29	66	130
		5:40	54	136
Average (first 7 observations)			113 ± 4; (σ 19)	
XVII	18.20	3:30	147	66
		3:36	127	72
		3:49	118	53
		4:08	136	56
		4:16	165	56
		4:21	230[3]	58
		4:34	221[3]	59
		4:42	169	55
		5:07	129	72
		5:14	145	65
		5:25	134	59
		5:32	153	60
Average			153 ± 6; (σ 34)	
Average (omitting 6th and 7th observations)			139 ± 3; (σ 16)	

TABLE 4—*Continued*

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	HEART RATE	
	kgm.		*cc.*		
XXII	17.5	11:52	221	69	
		12:03	270		
		12:11	221	74	Injection left heart
		12:20	175	72	
		12:52	265	72	
		1:07	166	72	
		1:11	235	72	
		1:26	203	69	
		1:31	214	73	Injection right heart
		1:39	175	80	
		1:45	185		
Average			212 ± 6; (σ 34)		
Average (first 7 observations)			222 ± 9; (σ 37)		
Average (last 4 observations)			194 ± 5; (σ 15)		
XII	15.25	3:40	199		
		3:49	192		
		3:57	156		
		4:11	191		
		4:20	218		
		4:35	207		
Average			194 ± 5; (σ 19)		
XXI	14.99	3:48	156	92	
		3:55	162	92	
		4:12	130	107	2% NaCl injected
		4:20	136	101	
		4:30	125	105	
		4:40	108	104	
		5:51	145	105	
		5:59	(187)[4]	104	1.5 %NaCl
		6:05	148	105	
Average			144 ± 5; (σ 22)		
Average (first 6 observations)			136 ± 5; (σ 20)		
Average (last 3 observations)			160 ± 7; (σ 19)		

TABLE 4—*Continued*

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	HEART RATE
	kgm.		*cc.*	
X	12.29		211	
			184	58
			200	58
			(325)[5]	58
			205	64
			225	69
			205	69
			232	65
Average (omitting 4th observation)			209 ± 3.8; (σ 14.8)	
XI	11.68	3:30	214	72
		3:52	185	70
		4:51	(244)[6]	73
		5:17	207	89
		5:30	210	108
Average			212 ± 6; (σ 19)	
Average (omitting 3rd observation)			204 ± 4; (σ 11)	
XX	10.32	10:35	225	64
		10:46	251	74
		11:04	257	80
		11:12	193[7]	72
		11:21	232	77
Average			232 ± 7; (σ 22)	
XVIII	9.89	3:38	164	66
		3:44	137	62
		3:56	164	62
		4:03	148	64
		4:08	131	58
		4:26	136	74
		4:36	(116)[8]	78
Average			142 ± 4; (σ 16)	
Average (first 6 observations)			146 ± 3; (σ 13)	
IX	9.29		283	
			277	
			297	
			236	
			281	
			(181)[9]	119
Average			(259)	
Average (first 5 observations)			275 ± 6; (σ 20)	

TABLE 4—*Concluded*

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	HEART RATE
	kgm.		*cc.*	
VIII	8.4		201	
			202	
XXIII	7.16	10:43	267	44
		10:51	235	37
		10:58	219	52
		11:15	269	49
		11:23	(163)[10]	48
Average			(211 ± 13)	
Average (first 4 observations)			247 ± 7; (σ 21)	
XV	4.97	3:00	317	82
		3:20	277	91
		4:45	331	74
		4:51	305	103
Average			307 ± 7; (σ 20)	

[1] Catheter somewhat obstructed by clot, so that only $\frac{1}{4}$ to $\frac{1}{3}$ of the usual quantity of salt solution ran into heart.

[2] Pulse rate abruptly increased; now unaffected by respiration (vagus paralysis). In the last observations pulse became weaker, and the circulation time from heart to femoral increased.

[3] Between 4:08 and 4:42 the output increased considerably and then diminished again (variations in anesthesia?).

[4] Pulse very strong. Time of injection only 8 seconds in this observation, as compared with 12 seconds in the others, and the quantity injected was probably too small for satisfactory measurement.

[5] This observation must be rejected owing to an error in resistance measurement of the sample. The data do not allow exact correction of the error, but the minute volume as given is certainly 50 per cent too high.

[6] The normal comparison sample for this observation was partially clotted, so that exact conductivity determinations could not be made.

[7] Collection finished too early, which would give too low an output as calculated by procedure II. The other observations were calculated on the rate at which the salt solution was injected (procedure I).

[8] The experiment was stopped by abnormally rapid clotting. The quantity of blood collected after injection of salt solution in this observation was too small for a fair sample.

[9] This observation should be omitted as it was made at the end, simply to test a technical point, a much smaller volume of a much stronger salt solution than was employed in the other observations of this experiment or in the other experiments being injected.

[10] The heart was failing at this point.

In experiments VIII to XIII and XV the animals were anesthetised by morphine and ether; in experiments XIV, XVII, XVIII, and XX to XXIII by morphine and ACE mixture.

to expect that the smaller animals should have a greater output per kilogram of bodyweight than the larger animals, in accordance with Rubner's results on the heat production in fasting dogs, which indicate a proportionality between the basal metabolism and surface area rather than between the basal metabolism and bodyweight. For this reason the experiments are arranged in the order of the bodyweights. Of course it would be desirable to have a much larger number of animals, especially at the two extremes, in order to test this point thoroughly.

It is well known that in experiments on different individuals of the same species made by the same method, the output per kilogram of bodyweight varies within wide limits. The variable response of different dogs to the experimental conditions, especially to the anesthetic, the differences in the build and breed of the animals and in the amount of fat entering into the bodyweight, as well as differences of age, size and other circumstances make it impossible to expect a close agreement in the average output of the different individuals in such a series as is given in the table. Even in non-anesthetised animals, as has been shown by Barcroft, Boycott, Dunn and Peters (9), and in man, as demonstrated by Krogh and his pupils (10), great variations occur. Muscular activity naturally exerts a great influence. The fact observed by Barcroft and his co-workers, that the proportion of the oxygen abstracted from the blood in the tissues may differ habitually in different individuals, must obscure any tendency to increase of output per unit of body weight in the smaller animals, until in a series of animals of progressively diminishing size the point is reached at which a physiological limit is set to any further abstraction of oxygen from, or accumulation of carbon dioxide in the blood. Above this point an animal with a habitually high coefficient of utilization may work with a lower cardiac minute volume than corresponds to its weight. But when the metabolism per unit of weight becomes several times as great in a small animal as in a large, it seems obvious that the minute volume of the heart must increase. Accordingly, a series of animals going down to quite small weights is most likely to reveal a relation between size and output per kilogram.

An important question is the degree of uniformity of successive determinations in the same animal, when no change is known to have occurred which is likely to affect the output. For the probable error of the average varies directly as the variability of the separate results and inversely as the square root of the number of observations dealt with. It will be observed that in regard to this criterion the averages deduced for

the separate experiments in table 4 must be considered satisfactory, although, of course, it would have been better to have a larger number of separate measurements from which to calculate the means. As Henriques justly remarks: "Eine Bedingung dafür dass die Methode benutzt werden kann, ist dass die injizierte Flüssigkeit tatsächlich mit dem Blut vermischt wird; dass dies mit Wahrscheinlichkeit stattfindet, hat Stewart in der obenerwähnten Abhandlung in hohem Grade wahrscheinlich gemacht, und die konstanten, mit der Methode gewonnenen Resultate, sprechen auch für eine solche Mischung."

It is all the more curious that Bock and Buchholtz (5), who speak with approval of the constancy of the results of Henriques (table 1), state that, "bei den schon erwähnten Untersuchungen von Stewart schwanken die bei demselben Tiere unmittelbar nacheinander ausgeführten Bestimmungen so weit voneinander, dass es sehr fraglich ist, ob man aus derartigen Einzelbestimmungen ein einigermassen richtiges Mittel erhalten kann." Their animals were anesthetised with a single dose of urethane and morphine at the beginning. The anesthesia was therefore constant during their short experiments. In my animals the anesthesia (morphine and then ether or ACE) was necessarily, and indeed purposely, more variable. Bock and Buchholtz cut the vagi, either at the beginning of the experiment or after one or two observations, and a good many of their animals were curarised. In my experiments the vagi were intact and no curare was given. The number of observations made by them on the same animal was generally much smaller than in our experiments. For all of these reasons it would not have been surprising if their individual observations did agree more closely. There is, however, little, if any real difference in this regard, as will be seen by comparison of table 4 with table 5, in which I have collected the measurements of Bock and Buchholtz and have calculated the averages with their probable errors and the standard deviation (σ). It will be noticed that these are precisely of the same order of magnitude as in my experiments (table 4). It should be explained that in many of the experiments of Bock and Buchholtz some change in the conditions was introduced between successive measurements (giving curare or section of the vagi, or administration of caffeine). But according to them none of these things affect the output, so that it did not seem worth while to swell the size of the table considerably by inserting them. The object of compiling it was to allow the reader at a glance to compare the results of the method in the hands of Bock and Buchholtz, under certain experimental condi-

TABLE 5

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	BLOOD PRESSURE
	kgm.		*cc*	
1	22.5	4:02	140	180
		4:13	138	187
2	29		187	142
3	10.5		144	141
4	32.7	4:16	176	133
		4:27	179	147
		4:42	192	161
Average			182 ± 2.7	
5	32.5	3:20	228	160
		3:40	209	137
		4:19	206	97
		4:32	204	115
Average			212 ± 3; (σ 9)	
6	11.2	3:40	163	154
		4:30	173	129
		4:45	169	140
		5:08	137	109
		5:27	89	55
Average			146 ± 9; (σ 31)	
7	9.1	3:33	169	143
		3:45	164	159
		4:21	127	139
		4:57	137	163
		5:20	140	148
Average			147 ± 5; (σ 16)	
8	26.5	4:25	143	140
		4:38	173	167
		4:59	165	172
		5:25	175	173
		5:53	129	109
Average			157 ± 5; (σ 18)	
9	25	3:42	102	134
		4:18	117	134
Average			109 ± 3	

TABLE 5—*Concluded*

EXPERIMENT	BODY WEIGHT	TIME	MINUTE VOLUME PER KILOGRAM	BLOOD PRESSURE
	kgm.		*cc.*	
10	25	3:50	234	151
		4:07	196	139
		4:19	228	137
		4:34	196	131
		5:02	188	117
Average			208 ± 6; (σ 19)	
11	17.5	3:42	159	140
		4:19	140	136
		4:36	127	125
		4:53	143	113
Average			142 ± 4; (σ 11)	
12	13	2:20	153	136
		2:30	161	128
		2:48	197	138
		3:12	161	135
Average			168 ± 6; (σ 17)	
13	19.8	3:54	114	164
		4:06	134	182
		4:25	179	176
		4:40	184	165
		5:20	194	120
Average			161 ± 9; (σ 31)	

tions, and in my hands under somewhat different conditions. It is clear that the results are substantially the same in the two sets of experiments. In the smallest dogs in table 4 there is a tendency for outputs somewhat higher than any in table 5 to appear. This is really a further rather striking confirmation of the general accuracy of the method since, as mentioned, we had a decidedly greater number of small animals than Bock and Buchholtz, and these small animals were generally not only small but not yet full grown. On the other hand, it would be idle to look in a series like this for a progressive increase in the output per kilogram as the body weight decreased. That would imply a uniformity of experimental conditions which is unattainable and a uniformity in the percentage of oxygen taken up by the blood

in passing through the lungs in the different individuals of the series which, as already mentioned, does not appear to exist (9). It is only feasible to compare the results, not only the averages but the extreme results, in fairly large groups of animals of very different size.

The averages deduced by Bock and Buchholtz by grouping their experiments in different ways also agree closely with mine, although they did not notice any special relation of output per kilogram to size. This could not be expected, as most of their 13 dogs were large and none really small. In 6 animals they found an average output of 157 cc. per kilogram per minute with intact vagi and without curare. Only one observation was made on each animal, except in one case where two were made. In the vagotomised dogs the average was 159 cc. per kilogram per minute. Comparison was not made in all cases in the same animal before and after section of the vagi. The variations in the output were from 179 to 114 cc. with intact vagi, and from 234 to 102 cc. after vagotomy. Their method of adding up all the separate observations and then taking the mean, instead of taking the mean of the average outputs for each dog, is objectionable, as it artificially weights any experiment in which more than one measurement happened to be made. No serious error, however, is introduced in the present instance, simply because only in one or two cases were even two observations made, the investigators usually contenting themselves with one measurement with intact vagi and one after vagotomy. As already mentioned, in a number of the animals no estimation of the output was made before the vagi were cut.

In their experiments on the curarised animals the output varied from 228 to 127 cc. per kilogram per minute; the mean, they say, lies "ein bischen" higher than in the non-curarised animals. As a matter of fact, if we average the 5 determinations in the way they did with the others, the average works out at 182 cc. per kilogram per minute. Even in the curarised animals considerable variations were found in estimations on the same animal, from 173 to 89 cc. per kilogram per minute in one experiment, the variation in this case being attributed by them to a change in the artificial respiration. In another animal, which was not curarised, but lightly anesthetised with morphine and urethane, the range was from 114 to 194 cc. per kilogram per minute. The chief part of the increase occurred after caffeine was administered, but the authors do not consider that caffeine has any definite effect upon the output, and attribute the marked variation in this case to the restlessness of the animal.

The highest output measured in their whole series was 234 cc. and the lowest 89 cc. per kilogram per minute. In all my observations on dogs weighing more than 10 kgm. (table 4), the greatest output was 270 cc. and the smallest 94 cc. per kilogram per minute, excluding one or two observations when the circulation was obviously failing. The greatest average output for these dogs was 232 cc., and the smallest 113 cc. per kilogram per minute.

This was also the smallest average output in the whole series. The animal (XIII) was a very fat spayed bitch weighing nearly 28 kgm. It ought really to be considered as a smaller animal carrying a great load of fat. This would artificially diminish the output per kilogram of normal bodyweight. Further, it is to be supposed that the metabolism was diminished by loss of the ovaries and the heat loss by the accumulation of fat. The organs (liver, spleen, kidneys, stomach and intestines) weighed, after bleeding, much less than the corresponding organs of other dogs of about the same weight. The heart also was probably somewhat small for the bodyweight. The thyroids were not enlarged. This is important to note in the case of dogs in the basin of the Great Lakes, as goiter is very common, accompanied by cardiac hypertrophy (goiter heart). This is best explained as a work hypertrophy associated with an increased cardiac output. The bloodflow through the thyroids per unit of mass is so great, and the circulation time from and back to the heart by this route so short that the additional work thrown on the heart by the presence of a large goiter might be as great as if an additional pair of hind limbs had been grafted on the neck. This is pointed out because in a series of dogs in this region the average output might perhaps be materially greater than in a corresponding series in a non-goiter region, even when animals with obviously large thyroids were rejected. While in pregnancy an increase of vascular territory is associated with an increase in blood volume (Spiegelberg and Gscheidlen, Rowntree et al.), it is not known, so far as I am aware, whether this is the case in simple goiter. The converse question, whether a great reduction in vascular territory in otherwise normal adults (after extensive amputations) is associated with reduction of blood volume, or whether the total average mass of the blood still retains its relation to the original dimensions of the body, has a certain general biological interest, and could probably be answered by means of the methods now employed for estimating blood volume in man.

The mean of the average outputs in the 6 dogs in my series weighing 15 kgm. or upwards was 156 cc. per kilogram per minute. Bock and Buchholtz, although one of their dogs weighed only 9.1 kgm., used mostly large animals, 9 out of 13 weighing from 17.5 to 32.5 kgm. The average weight of their 13 animals was 21.1 kgm.; the average weight of my 14 dogs, 14.2 kgm. The mean of the average outputs in my series of the 8 animals weighing 12.3 kgm. or less was 225 cc. per kilogram per minute. For the whole 14 dogs the mean of the average outputs was 195 cc. per kilogram per minute.

Treating the figures in another way, we get for 126 kgm. of animals (the 6 weighing 15 kgm. or more) a combined average output of 20.02 liters per minute, i.e., 159 cc. per kilogram per minute; for the 8 smaller dogs, weighing together 74.1 kgm., a combined average output of 15.97 liters per minute, i.e., 215 cc. per kilogram per minute. For the 14 dogs, weighing together 200 kgm., the combined average output was 36 liters per minute, or 180 cc. per kilogram per minute.

Bock and Buchholtz consider that the recent results of Barcroft and others (9) on unanesthetised goats, weighing from 14 to 38.6 kgm. (mean output 133 cc. per kilogram per minute for 21 animals; extreme range of the means 220 to 55 cc.) agree quite well (recht gut) with their own results on anesthetised, curarised and vagotomised dogs of about the same weight. The average weight of Barcroft's goats was 23.67 kgm., considerably more than that of the dogs of Bock and Buchholtz, and much more than the average of my dogs. One goat weighed 14.1 kgm., 4 from 17.3 to 19.5 kgm.; all the others were over 20 kgm. The largest single measurement of the minute volume was 220 cc.; the smallest, 46 cc. per kilogram.

Gréhant and Quinquaud (11) determined in 6 dogs, weighing from 6 to 18 kgm., the carbon dioxide content of blood obtained simultaneously from the right heart and the carotid artery, and also the amount of carbon dioxide given off per minute from the lungs, from which the quantity of blood passing through the lungs is calculated. Theoretically the arterial sample should be taken slightly later than the venous sample (at an interval equal to the circulation time from right ventricle to carotid). They apparently made only one determination on each dog. They do not give the bodyweights, but on the assumption that the smallest flow per minute per animal belongs to the smallest dog and the largest blood flow to the largest dog, we get 84 cc. per kilogram per minute, and 145 cc. per kilogram per minute respectively, for these two animals. But from the way the results are reported in the very brief note, this can only be a guess. It is therefore difficult to see on what grounds Bock and Buchholtz state that the results of Gréhant and Quinquaud appear to agree quite well (recht gut) with their own, whereas the observations of Zuntz were made with such an inadequate method that they can scarcely now be considered reliable. Zuntz used more than one method and one of these (12), in which the quantity of liquid which must be injected into the aorta during stoppage of the heart by the vagus in order to maintain the blood pressure at the original level is taken as equal to the output, can certainly not be con-

sidered adequate. But this criticism does not apply to Zuntz's experiments, carried out on the same principle (Fick's principle) as those of Gréhant and Quinquaud, but with estimation of the oxygen instead of the carbon dioxide. He found an output of about 73 cc. per kilogram per minute in a resting horse weighing 360 kgm., and about 90 cc. per kilogram per minute in resting dogs weighing 25 kgm. The observations of Barcroft and his collaborators were made by a similar method.

SUMMARY AND DISCUSSION

The recent results of Bock and Buchholtz (5), obtained by my method of estimating the output of the heart (but with injection of sodium iodide) are shown to agree well with the results obtained by the same method, with injection of sodium chloride. The two procedures according to which the method may be applied are discussed.

The first procedure, involving the collection of a sample of the blood mixture after the maximum concentration of the injected salt has been reached, and while the concentration remains steady at the maximum, was employed by the writer in the greater number of his observations, and later by Bock and Buchholtz. The quantity of blood entering the heart and mingling there with the injected salt solution is determined from the formula $V = Q\frac{60}{mT}$, where V is the volume of blood coming to the heart in a minute, Q, the volume in cubic centimeters of salt solution injected in T seconds and m the volume in cubic centimeters of the salt solution which must be added to 1 cc. of the normal blood sample taken before injection of the salt solution, in order to make its conductivity the same as that of the sample collected during passage of the blood mixture.

The second procedure was used in some of my observations and later by Henriques (4) (with injection of sodium sulphocyanide, instead of sodium chloride). It involves the collection of a sample of the blood mixture for the whole time during which it is passing the point of collection. On the assumption that the cardiac output remains approximately constant during the short time of an observation, no error is involved in commencing the collection at the moment when the injection of the salt into the heart is begun, as was done by Henriques. The output is calculated from the formula $V = Q\frac{60}{mt}$, where V is the minute volume, Q, the volume of salt solution injected in cubic centi-

meters, m, the volume of the solution which must be added to 1 cc. of the normal sample to make its conductivity the same as that of the sample collected during passage of the blood mixture, and t, the time of collection of the blood.

Any error dependent upon faulty collection with procedure I is most likely to give too high a value for the minute volume, and the opposite with procedure II. For in procedure I it is the maximum concentration which is to be determined. We cannot collect so as to obtain a higher concentration, but may in error start collection too soon or continue it too long. In either case a lower average concentration than the maximum would be given by the specimen, and therefore a value higher than the correct one would be calculated for the minute volume. With procedure II, if collection is begun before the arrival of the blood mixture no error results, but if it is begun slightly later than the arrival of the mixture and stopped before it has completely passed, a higher average concentration is obtained than if the sample is collected during the entire time of passage. The calculated minute volume will then be too low. So far as the avoidance of these errors is concerned, there may be some advantage in collecting a number of small successive samples during passage of the blood mixture. These can then be examined at leisure.

The general agreement in the results of the method in the experiments of Bock and Buchholtz and in those of the writer, although different substances were employed for injection, seems to indicate that it can be considered satisfactory for the study, in acute experiments on anesthetised animals, of the changes which the minute volume undergoes under the action of various factors, for instance, under the influence of drugs. A relatively large number of observations can be made on the same animal. It is essential to distinguish accidental changes, due to variations in the anesthesia or to respiratory or other movements, or to general deterioration of the animal with alterations in the blood pressure, from genuine effects of the factor under investigation. This may sometimes be difficult or impossible, and then the experiment must be rejected. The principle of the method is essentially the same as that originally suggested by Fick and used by Zuntz (13), and recently by Barcroft and his co-workers (9) in their experiments on unanesthetised goats, but instead of oxygen being added to the blood in the normal process of respiration, sodium chloride or some other substance is artificially added. The method is, of course, inferior to that employed by Barcroft in that it cannot easily be used

without anesthesia. It is perhaps barely possible that a satisfactory maximum concentration could be estimated by injecting into a vein through a needle, as now practised without anesthetics, a substance (vital red, e.g., or a drug used for intravenous medication) and collecting successive small specimens of blood by puncture of an artery, a procedure now also used to some extent in man (or in suitable animals by heart puncture). But even if possible, it would be quite another question whether such a method could be considered seriously in comparison with the gasometric methods. Whether with the refined technique introduced by Haldane, Barcroft and others for estimating blood gases and the respiratory exchange, gasometric methods would also be better even for observations on anesthetised animals, could only be decided by experiment, and perhaps the degree of experience of the individual investigator might be a matter of some importance.* Henriques states that he employed the injection method because he found the methods of Krogh and Lindhard not useful for animals. Theoretically, there is one point of superiority in the injection method as compared with Zuntz's method, namely, that the quantity of the substance added to the blood per unit of time is accurately determined, even for a period of a few seconds, by a simple volumetric reading. On the other hand, the addition of liquid to the blood, except in so far as this may aid in compensating for the blood withdrawn, is a drawback from which Zuntz's method is free.

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* Since this was written experiments on cats under urethane have been published by one of Barcroft's pupils. With the uniform anesthesia the values obtained for the minute volume in successive observations were admirably concordant.

STUDIES ON THE SECRETION OF THE PARS PLYORICA GASTRI

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During the course of a series of studies on gastric ulcer one of us (1) devised an operation by which the pyloric portion of the stomach was isolated as a pouch with or without nerve supply intact. On making an analysis of the secretion of this pouch, our results showed discrepancies when compared with the reports in the literature and textbooks concerning the character of this secretion. Therefore an attempt toward a complete study of the physiology and character of the secretion of the pyloric portion of the stomach has been made.

Method. The operation for the production of the pouch will not be described here as it has been described in detail elsewhere (1).

The secretion was collected in a glass beaker enclosed in a copper cup (shaped like a silk hat) which was held in place about the opening of the pouch by means of a bandage.

All observations have been made on healthy animals, both immediately after operation and at periods up to as much as 18 months following operation. The nutrition of the animals is not disturbed in any way provided liquid and macerated food is fed for 3 to 4 weeks following the operation. Bones should not be allowed at any time as they have caused obstruction, not only at the orifice of the gastroenterostomy but as far down the intestine as 5 feet, in several animals of our series of over fifty successfully operated.

We believe that the secretion as we collect it from the pouch with or without the nerve supply intact is the normal secretion of the pyloric mucous membrane just as the secretion that is collected from the Pavlov pouch is the normal secretion of the fundic portion of the stomach. The mucous membrane is normal in appearance, being of the same color as the mucous membrane of the Pavlov pouch. Histological studies of the mucous membrane that were made and that will be referred

to later in the paper prove that the mucosa of the pouch is histologically normal. Studies on the absoprtion of the mucosa of the pyloric pouch, which are to be reported in a later communication, show the rate of absorption to be within the normal variations of the rate of absorption of the mucosa of the pyloric antrum isolated in situ by means of ligatures so placed as not to interfere with the normal blood supply.

PHYSIOLOGY OF THE SECRETION

Character of the secretion. The secretion is mucoid, viscous, tenacious, transparent, odorless and slightly salty in taste. In appearance and consistency it is best compared with egg white. On standing from 3 to 4 hours, or on centrifuging, a thinner, yet viscous, portion separates and rises.

Amount of secretion. In amount our observations compare favorably with those of Heidenhain (2), who reports a secretion of from 2 to 3 cc. per hour.

We find that the secretion varies in amount from 1 to 5 cc. per hour. The average secretion per hour for ten dogs with the nerve supply of the pouch severed is 2.25 cc., and for five dogs with the nerve supply of the pouch intact is 3 cc. We attach no significance to this slightly larger amount in the case of the pouches with the nerve supply intact. The secretion is more or less uniform in amount, but varies in different dogs and in the same dog from day to day.

In two animals which have been kept for 18 months following the operation the amount of secretion per hour in one of them is the same as it was a few weeks after the operation, in the other the amount of secretion per hour is about 75 per cent of the original amount. However, in the mucosa of the pouch of the second animal four experimental ulcers have been produced with resulting scar formation which might account for the diminution in the amount of the secretion.

Influence of meals and water drinking on the quantity of the secretion. Meals and water drinking do not influence the amount of the secretion, which is true for pouches with or without the nerve supply intact.

Heidenhain (2) reports that the secretion is slightly increased following the meals and reaches its maximum about the 5th hour. We were unable to confirm this report in a long series of experiments as any increase that occurred came within the normal variations of the rate of secretion and there was no constancy in the changes in the rate of secretion following a meal.

Diet does not influence the quantity of the secretion. Deprivation of water diminishes the amount of secretion, but the effect is not noticeable until after 24 hours.

Influence of "gastrin" and secretin on the quantity of the secretion. Proven active preparations of gastrin and secretin when injected subcutaneously do not increase the amount of the secretion or alter it in any way.

When gastrin is fed to the animals no increase in secretion results. (One hundred cubic centimeters were fed.) When gastrin is applied to the mucosa of the pouch the rate of secretion is not altered.

Influence of acid and of "fundic" secretion on the quantity of the secretion. The application of N/10 HCl or of gastric secretion from a Pvalov pouch to the mucosa of the pouch for a period of 5 minutes augments the hourly secretion from two to three times. On the application of either of these substances to the mucosa of the pouch hyperemia or congestion of the mucosa occurs, which probably accounts for the increase in the amount of the secretion. If the mucous membrane is gently massaged for 15 to 20 minutes with a cotton sponge soaked with N/10 HCl, it begins to bleed, the blood apparently oozing out instead of coming from some visible excoriated area. If gastric secretion from a Pavlov pouch is used instead of acid, bleeding does not occur as readily even though the titratable acidity is as great as that of the N/10 HCl. This bleeding only occurs on the outer or exposed edge of the pouch where the mucosa is subjected to more trauma (no visible lesions being present) and to the removal of the mucus secreted. The acid or gastric juice does not cause bleeding when applied for a long time to the unexposed portion of the pouch even though the same amount of massage is used.

Reaction of the secretion. The secretion reacts slightly aklaline, giving a pH varying from 7.00 to 7.50 when measured by the gas chain and colorimetric methods. It is neutral to litmus in most instances.

Heidenhain (2), Klemensiewicz (3) and Akerman (4) reported the secretion to be alkaline. Contejean (5) reported the secretion to be acid and explained the results of the first two investigators as due to atrophy of the mucosa of the pouch. This explanation is hardly tenable for we (6) have made histological study of the mucous membrane of the pouch and find no change even after 10 months. Contejean's findings of an acid secretion can be easily explained by a possible inclusion of some fundic mucous membrane in the pouch he made.

Is pepsin present in the secretion of the pyloric mucous membrane? Edkins (7) states that "it may be regarded as established that the pyloric glands do secrete pepsin." His statement is based on the physiological observations of Heidenhain (2), Klemensiewicz (3), Akerman (4), Contejean (5), Bergman (8), Ebstein and Grutzner (9) and Langley (10) and on the histological observations of Nussbaum (11), Langley (12), Stohr (13) and Oppel (14). Klug (15), Glaessner (16), Wassmann (17) and Wittich (18) report that any pepsin that was present in the pyloric mucous membrane was due to infiltration or absorption. All of this early work is controversial and is open to criticism in light of modern methods. Later histological investigation by Bensley (20) and Harvey (21) shows that neither parietal nor ferment cells are present in the mucosa of the pyloric portion of the stomach and that the line of demarcation between fundic mucosa and pyloric mucosa is rather sharply defined. Bensley (19) states that "the theory of Heidenhain, that the cells of the pyloric glands are pepsin-forming elements similar in character to the chief cells of the body of the fundus gland, has been shown to be incorrect" and that histological evidence indicates "that the bulk of the secretion of the pyloric gland is simply mucus." In our histological study of the pyloric mucous membrane we have failed to find either parietal or chief cells.

In a preliminary report by one of us (22) it was stated that a small amount of pepsin was present in the secretion. In the first five dogs operated we obtained peptic digestion of from 0.5 to 1 mm., using Schiff's modification of Mett's method. We also noticed a slight increase in peptic activity of the secretion in the 3rd and 4th hours after a meal and by the subcutaneous injection of gastrin. Since making the preliminary report the secretion of twelve other dogs has been examined for pepsin with negative results. We believe that the presence of pepsin in the secretion of the first five dogs operated was due to the inclusion of some fundic mucosa in the pouch due to insufficient care in the separating the pyloric portion from the fundic portion of the stomach. We believe that the presence of pepsin in the secretion of the two or three dogs reported by Heidenhain (2) can be explained in the same way. Akerman's technique for making the pyloric pouch can hardly be criticised. He only had one dog to live, however, and his method for determining the presence of pepsin is open to criticism in the light of modern methods and knowledge.

We also fail to find pepsin in the secretion from a pouch whose nerve supply is intact, which answers a possible criticism that the absence of

pepsin is due to the severing of the nerve supply, which objection has been raised by Matthews (23) against Heidenhain's observations.

The secretion has been examined immediately after the operation and at intervals up to as long as 18 months after the operation, pepsin being found at no time.

Is pepsinogen present in the pyloric mucous membrane? The mucous membrane of the pouch was treated with N/10 HCl and also with inactivated gastric juice for 5 minutes to ascertain if the contact of the HCl or inactivated gastric juice might not activate a possible pro-enzyme in the cells of the pyloric mucosa. As a result of this procedure the secretion was increased in amount from two to three times and rendered less viscous, but no peptic activity was present.

Also, as pointed out before, when the pyloric secretion itself is treated with N/10 HCl for activation of a possible pepsinogen, no digestion resulted in Mett's tubes.

TABLE 1

Showing effect of pyloric secretion on peptic activity

	SERIES A DIGESTION IN MILLIMETERS	SERIES B DIGESTION IN MILLIMETERS
First day	1.52	1.57
Second day	1.72	1.70
Third day	1.52	1.53
Fourth day	1.82	1.70
Fifth day	1.52	1.56
Average for 5 days	1.62	1.61

Effect of pyloric secretion on the peptic activity of fundic secretion. To ascertain the effect of pyloric secretion on the peptic activity of the fundic secretion two series of tubes were set up. One, A, containing 1 cc. of fundic secretion (obatined from a Pavlov pouch), 1 cc. of pyloric secretion and 15 cc. of N/10 HCl; the other, B, containing 1 cc. of fundic secretion, 1 cc. of distilled water and 15 cc. of N/10 HCl. To each series Mett's tubes were added. Both series were incubated at 37.5° C. for 24 hours. The pyloric secretion of two dogs on five successive days was used. The results are shown in table 1 and demonstrate that the pyloric secretion has no effect on the peptic activity of the fundic secretion. In other words, *the pyloric secretion does not contain an antipeptic ferment.*

Is there a proteolytic enzyme present in the secretion other than pepsin?

Even though the secretion does not contain pepsin in any form, it is possible that it might contain a proteolytic enzyme similar to erepsin or trypsin acting in an alkaline medium.

By using Sorensen's method for erepsin and trypsin with large quantities of pyloric secretion (10 cc.) we were unable to find any evidence of the presence of either erepsin or trypsin in the secretion. The tests were carried out under absolute aseptic procedure, using toluol-treated secretion. If the secretion was not treated with toluol, digestion always occurred, as would be expected as the secretion is teeming with bacteria.

Is diastase present in the secretion? Klemensiewicz (3) reported that the secretion contained diastase. Heidenhain (2) failed to find it.

In our examination for diastase the secretion was treated with toluol and sterilized apparatus was used to prevent bacterial action. To 4 cc. of sterile starch paste solution 1 cc. of toluol-treated secretion was added. The reaction of the mixture was slightly alkaline (pH 7.5) in one series, neutral in another and distinctly acid to litmus (N/40) in another. The mixture was then kept at 38°C. for ½ hour and incubated for 3 hours at 38°C. At the end of this time a trace of dextrin was found when compared with the control.

We doubted that this trace of diastase was a product of the cells of the mucous membrane; but rather thought that it was only a filtrate from the blood. To determine this we injected intravenously Takadiastase and found the diastase content of the secretion to vary directly with the amount of diastase injected. Hence we are of the opinion that the trace of diastase in the pyloric secretion is from the blood and is not formed by the cells of the pyloric mucous membrane.

Is invertase present in the secretion? Klemensiewicz (3) reported that invertase was present in the secretion. His secretion was mixed with pus, so his results are to be questioned.

All of our observations, made under antibacterial and sterile procedure, failed to demonstrate the presence of an invertase. One series of tests was made with the reaction of the mixture of saccharose solution and secretion slightly alkaline (pH 7.3) and another series with the reaction distinctly acid to litmus (N/40 HCl).

Is maltase present in the secretion? All of our tests for the presence of maltase in the secretion were negative. The same precaution of asepsis was taken and the importance of the reaction of the mixture was considered as was done in the case of diastase and invertase.

Is lipase present in the secretion? We were unable to detect any lipase in the secretion when antibacterial procedure was followed. The litmus milk and litmus olive oil tests were used.

Is rennin present in the secretion? Heidenhain (2), Klemensiewicz (3), Akerman (4) and Contejean (5) reported that rennin is present in the secretion of the pyloric mucous membrane.

In all of our observations we were unable to demonstrate the presence of rennin when 1 cc .of the secretion was incubated at 37° C. with 5 cc. of milk for periods of from ½ to 3 hours. The secretion in another series of tests was activated by incubating the secretion with N/10 HCl for periods varying from 15 minutes to 1 hour and then neutralizing it with sodium bicarbonate solution. This activated secretion failed to cause coagulation. As much as 5 and 10 cc. of the secretion were used with the same negative results.

The influence of pyloric secretion on the coagulation of milk by rennin was studied. It was found that the secretion retarded slightly the coagulation of milk by rennin, but not any more, however, than the same amount of distilled water. The clot formed in the presence of pyloric secretion is less compact than the clot formed in the absence of the secretion. This was also found to be true in the coagulation of milk by gastric juice from a Pavlov pouch, i.e., if pyloric juice was added to the gastric juice, the resulting clot was less compact than with gastric juice alone.

It is to be recalled that Heidenhain, Akerman and the above mentioned investigators found pepsin in the secretion of their pouches, hence rennin would also be expected. We explain the presence of rennin and pepsin in the secretion of the pouches of the above investigators as due to the inclusion of some fundic mucous membrane in the pouches they made. Contejean found acid along with the pepsin and rennin. Heidenhain, Ackerman and Klemensiewicz found pepsin and rennin but no acid. Our explanation as to why they did not get acid associated with the pepsin and rennin is that physiologically their animals may have been in such a condition of health that no acid was formed. We met this possible objection by making five two-gastric-pouch-dogs, consisting of a pouch of the pyloric antrum and a Pavlov pouch, and collecting the secretion of the two pouches simultaneously. The Pavlov pouch gave the characteristic highly acid-pepsin-rennin secretion while the pyloric pouch gave the characteristic secretion described in this paper.

Anaphylaxis and precipitin formation? Two rabbits were injected intravenously on alternate days with 5 cc. of the secretion for a period of 2 weeks, following which the animals' serum was examined for a precipitin of pyloric juice, with negative results. An attempt was also made to sensitize two guinea pigs and a rabbit against the juice, with

negative results. The technique used by Elliot (25) was followed for both precipitins and anaphylaxis.

The speculative purpose of these experiments was to determine if the juice contained a protein that would cause antibody formation. Wells (24) states that any enzymatic secretion injected results in the formation of antibodies. Elliot (25) reports that mucin is an anaphylactogen. He prepared the gastric mucin he used by extracting the mucosa of the pig's stomach, while we worked solely with the secretion of the mucosa. Carlson (26) was not able, however, to sensitize an animal against human gastric juice.

Autodigestion? If the secretion is allowed to stand in an incubator for several hours, it becomes less viscous and reacts more acid. Such a change suggests a possible autodigestion as reported by Glaessner (16). But when toluol-treated secretion is incubated for 4 hours the above changes do not occur and there is no change in the color of the biuret reaction, or in the amount of alcohol precipitable mucin or proteins. So the liquefaction and change in reaction of the secretion on standing are most probably due to bacterial action, as microscopically many bacteria are present in the secretion.

Is secretagogue present in the pyloric secretion? Ten cubic centimeters of the fresh pyloric secretion have been injected a number of times both intravenously and deep intramuscularly into a Pavlov pouch dog without stimulating or having any effect on the secretion of the Pavlov pouch. Also 100 cc. of the clear pyloric secretion were incubated with 100 cc. of N/10 HCl and extracted as in the preparation of gastrin. Ten cubic centimeters of this extract when injected intramuscularly failed to cause any effect on the gastric secretion of a Pavlov pouch dog. Further, 100 cc. of the clear pyloric secretion was incubated with 100 cc. of gastric juice collected from a Pavlov pouch and extracted as in the preparation of gastrin. This extract also failed to cause any effect when injected. One hundred cubic centimeters of pyloric secretion were given to a Pavlov pouch dog by mouth without any stimulation of gastric secretion resulting.

This evidence is interpreted as showing that the secretion does not contain a secretagogue. Koch, Luckhardt and Keeton (27) showed this to be true for fundic secretion from a Pavlov pouch which makes the conclusion possible that none of the external secretions of the gastric mucous membrane contain secretagogues.

CHEMISTRY OF THE PYLORIC SECRETION

The results of chemical analyses up to date are shown in table 2. All analyses have been made upon clear secretion free from cellular detritus. The results reported (table 2) are the maximum and minimum found for the secretion of six different dogs. The analyses are expressed in grams per 100 cc. of secretion.

The specific gravity of the secretion as observed by us compares with that reported for the secretion by Klemensiewicz (3) (1.009 to 1.010). The osmotic pressure is slightly greater than that of the blood and "fundic" gastric juice of the dog as reported by Carlson (25). In this connection attention should be called to the chloride content of the secretion, which is also slightly higher than that of blood and "fundic" gastric juice, the average of our figures being 0.632 gm. per 100 cc. of secretion. It is interesting to note that the amino-acid nitrogen is the most constant of the substances of the analyses.

TABLE 2

*Showing the results of the chemical analyses of pyloric secretion grams, per 100 cc. of secretion**

	MAXIMUM	MINIMUM
Total solids	2.420	1.331
Total ash	1.540	0.558
Total nitrogen	0.095	0.054
Alcohol precipitable substances	1.120	0.896
Total chlorides	0.640	0.458
Total NH_2-nitrogen	0.0054	0.0033
Total amino-acid nitrogen	0.0025	0.0023
Specific gravity	1.011	1.008
Degrees of depression of freezing point	−0.640	−0.610

The biuret reaction gives a dark purple color.

* Only centrifuged samples were used.

SUMMARY

The secretion of the mucous membrane of the pyloric antrum is mucoid, viscous, tenacious, transparent, odorless and slightly salty in taste. The rate of formation varies from 1.0 to 5.0 cc. per hour and is not increased by meals, water drinking or secretagogues. Acids and irritants when applied to the mucous membrane excite the formation of the secretion. The secretion is alkaline (pH 7.00 to 7.50) which confirms

the reports of Heidenhain, Klemensiewicz and Akerman. The secretion does not contain enzymes, which is contrary to the reports of Heidenhain, Klemensiewicz, Akerman and others. The findings of enzyme by the early investigators is explained by faulty operative technique, the presence of pus in their secretion, bacterial enzymatic action and inadequate methods for determining enzymatic activity. The secretion does not contain an antipeptic ferment and does not form antibodies when injected parenterally. A gastric secretagogue is not present in the secretion. The results of chemical analyses are reported.

Hence our studies on the physiology of the pyloric secretion up to the present time substantiate the conclusion reached by Bensley (19) from a histological study of the cells of the mucosa of the pyloric antrum that "the secretion of the pyloric gland is simply mucus"; and we further add that it contains no substance that is of specific digestive importance.

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STUDIES IN NUTRITION

IX. The Nutritive Value of the Proteins from the Chinese and Georgia Velvet Beans

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Chemical studies of the proteins of the Chinese and Georgia velvet beans have already been reported from this laboratory (1), (2), (3). The results obtained indicated that the amino-acid content of the proteins from these seeds was biologically adequate. When the cooked or autoclaved bean meal, however, furnished the only source of protein in an otherwise complete diet and was fed to albino rats, there was little or no growth. The addition of cystine or casein did not improve the diet in this respect. A ration prepared from the protein obtained by dialysis[1] was also inadequate for normal growth. The animals on these diets all showed signs of malnutrition. Their fur was ragged and their diets apparently caused regurgitation and diarrhea. That it is a question of digestibility or toxicity is indicated by our nutrition experiments with the protein obtained by heat coagulation[2] from either the Chinese

[1] The dialyzed protein was prepared by dialyzing in running tap water the clear 10 per cent sodium chloride extract of the bean meal. The protein which precipitated was washed with distilled water until free from chlorides. The protein was dried by treating successively with absolute alcohol and absolute ether. The ether was removed by heating the protein in a vacuum oven for a few hours at 110°C.

[2] The coagulated protein obtained by heating a clear 10 per cent sodium chloride extract of the meal at 105°C. until no more protein coagulated, was collected on a folded filter. The coagulum was suspended in distilled water and washed by decantation until free from chlorides. It was dehydrated by treatment with absolute alcohol and finally with absolute ether. The latter was removed by drying for a few hours at 110°C.

Both the dialyzed and the coagulated proteins are mixtures of two globulins differing chiefly in their sulphur and nitrogen content as well as their solubility in ammonium sulphate solutions.

or Georgia velvet bean. The protein prepared in this manner and supplemented with the other necessary dietary ingredients produced normal growth when fed to albino rats.

Miller (4) has isolated free dihydroxyphenylalanine from the seeds of the Georgia velvet bean. This amino acid has been reported to cause vomiting, and its presence probably accounts for the nutritional disturbances found in our experiments with the cooked or autoclaved bean meal. This would not account, however, for the failure of the rats to grow on the dialyzed velvet bean proteins.

It has previously been shown, in the case of the navy bean, that its proteins require cooking (5) in order that they may become available for the normal growth of albino rats, and that the improvement thus secured is probably due to an increase in digestibility (6). Preliminary experiments, made by H. C. Waterman of this Laboratory in a study of the digestibility *in vitro* of the velvet bean proteins, make this explanation the most probable one. The protein prepared by dialysis from the Chinese velvet bean was digested but little more than one-half as much as was that made by coagulation from the same bean. The figure obtained from the coagulum agrees well with those given by *cooked* phaseolin (6) and by casein. The good growth secured with the coagulated protein could then be explained as due to an increased digestibility, resulting from the boiling, in faintly acid solution, which was necessary to effect coagulation. It should be borne in mind, however, that a protein toxicity or an adsorbed toxic substance might also account for the failure of the dialysis product; and that the one might have been destroyed or the other removed in the preparation of the coagulum.

Scott (7), (8) has reported that ground velvet bean feed, containing both the seeds and pods, could replace an equivalent amount of peanut meal in a ration fed to cattle with no apparent ill effects; when fed to pigs results were obtained in some cases which indicated that it may cause abortion. Sure and Read (9), in a preliminary announcement of their experiments, suggest that the Georgia velvet bean is toxic in high concentration, impairing both growth and reproduction. They also state that the proteins from this seed were biologically deficient.

Experiments with cooked and autoclaved Georgia velvet bean meal. A diet prepared from cooked[3] Georgia velvet bean meal supplemented

[3] A thin paste was made by adding distilled water to the finely-ground beans. This was then cooked for 2 hours in a double boiler. The cooked meal was dried at a temperature of 60°C. and again ground to a fine powder, which was incorporated in the experimental diets.

by the addition of cystine,[4] Osborne and Mendel's salt mixture, butter fat and lard, was fed to albino rats. After a short time on this diet the animals became sickly in appearance, their fur was ragged and but very little growth occurred. The regurgitated velvet bean meal produced dark stains under their bodies. This may have been due to the oxidation of free dihydroxyphenylalanine in the regurgitated velvet bean meal; Miller (4) found that the aqueous extract of the bean meal darkened on exposure to the air, and it was shown that this darkening was

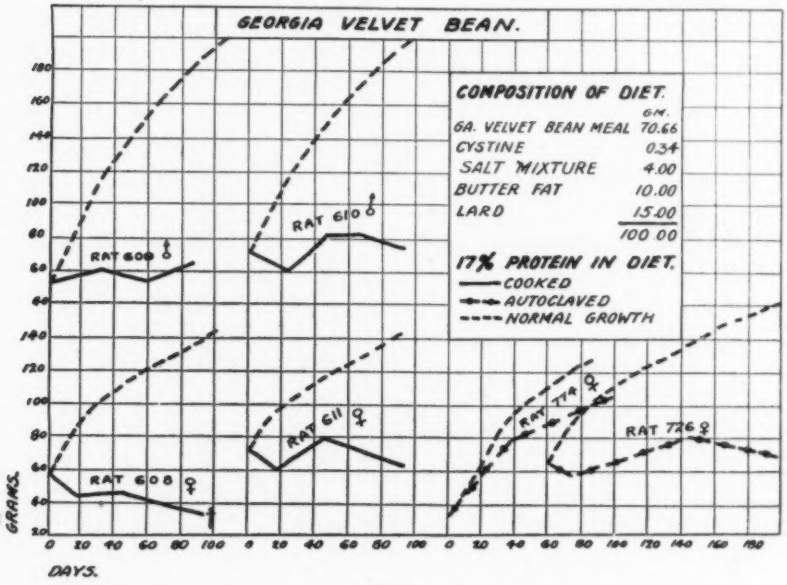

Chart 1

due to the oxidation of dihydroxyphenylalanine. There was a slight improvement in appearance of the animals when fed a diet similar except that the bean meal was autoclaved[5] at 15 pounds for one hour. The composition of these diets and growth curves are shown in chart 1.

[4] The cystine used in these experiments was prepared by Mr. S. Phillips of the Protein Investigation Laboratory, Bureau of Chemistry.

[5] The autoclaved bean meal was dried at 60°C. after removing from the pressure cooker, and reground to a fine meal.

Experiments with cooked and autoclaved Georgia velvet bean meal supplemented with casein. In order to eliminate the possibility of deficient proteins, casein was incorporated in the diet of cooked velvet bean meal, to which the other necessary dietary ingredients were added. No appar-

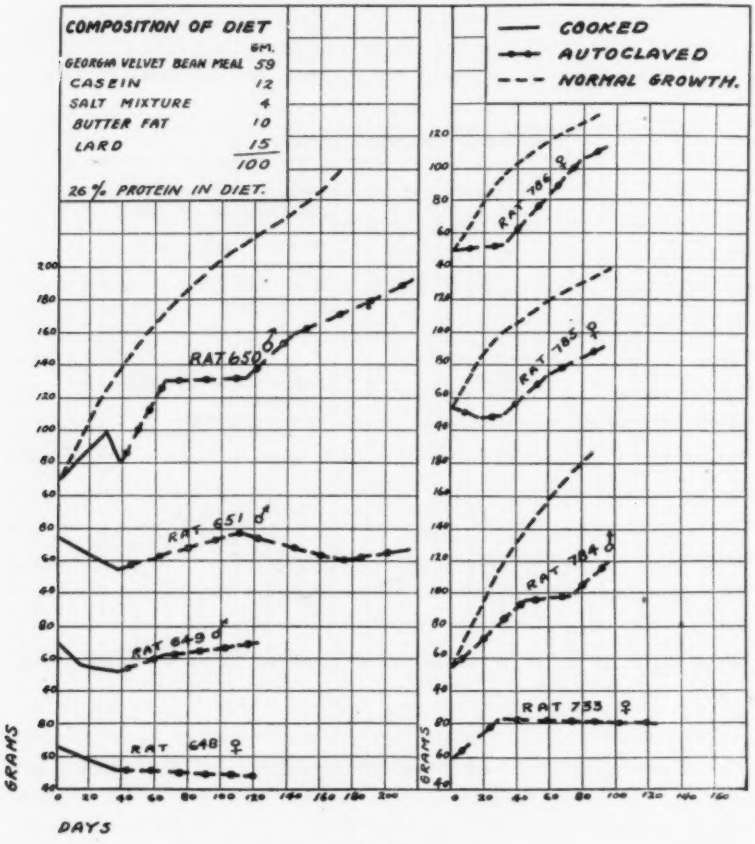

Chart 2

ent change was noted between the group fed the diet containing casein and those not receiving the additional protein. With a similar diet in which autoclaved meal was fed, it was noted that while the rats did not grow at the normal rate, regurgitating did not occur. The appear-

ance of the animals in this group was also noticeably better. The results of these experiments indicate that some toxic substance was responsible for the regurgitation and, in part at least, for the lack of growth. Heating apparently improved the diet with respect to the former disturbing factor. The results of these experiments are shown on chart 2.

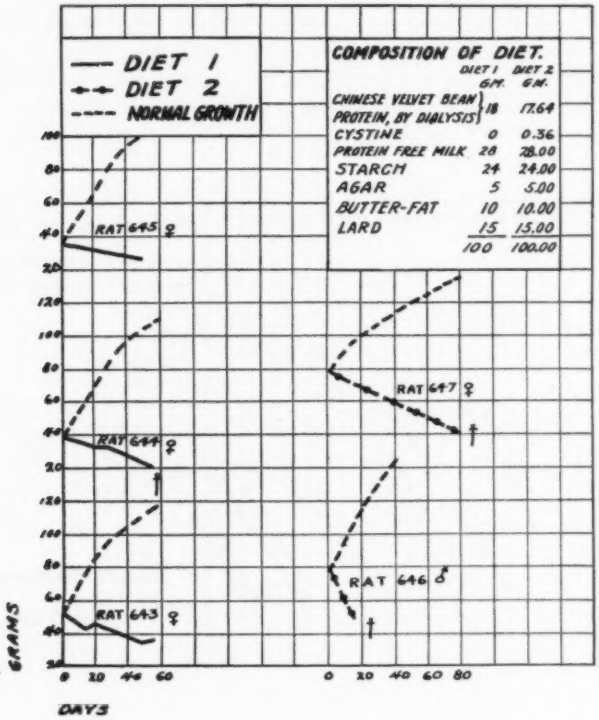

Chart 3

Growth experiments with the isolated protein from the Chinese and Georgia velvet beans. A number of experiments were made with the isolated protein from the Chinese velvet bean which was prepared by dialysis. If a toxic substance present only in the seeds but not necessarily in a combination with the protein was the disturbing factor, then a diet prepared with isolated protein, to which was added the other necessary dietary ingredients, should eliminate the question of toxicity. The

animals placed on such a diet, however, lost rapidly in weight although their food intake was normal. Previous experience with the navy (5), adsuki (10) and lima (11) beans had shown us that the proteins of these seeds were deficient in cystine. Addition of cystine, however, did not improve the results obtained from the dialyzed velvet bean protein diet; the animals lost rapidly in weight and died.

Entirely different results were obtained, on the other hand, when the protein prepared by coagulation with heat was fed. Normal growth occurred when the source of proteins[6] was either from the Chinese or Georgia velvet beans. The addition of cystine was not required.

The growth curves and composition of the diets are recorded on charts 3 and 4.

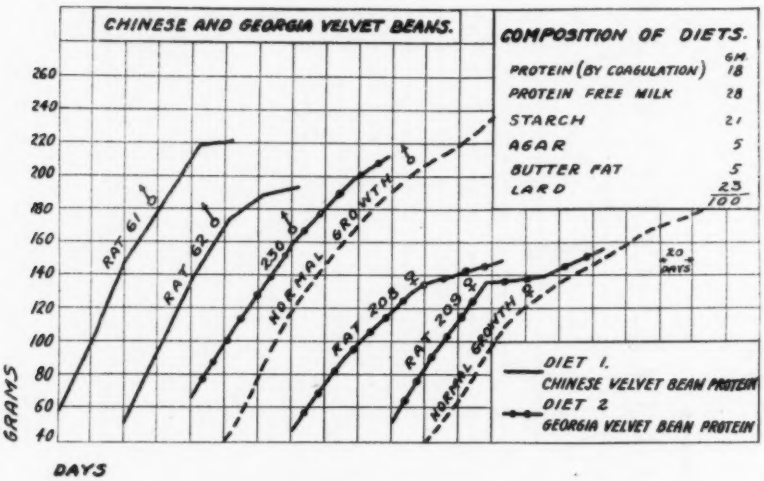

Chart 4

SUMMARY

1. The isolated proteins obtained by coagulation with heat from either the Chinese or Georgia velvet beans are adequate for the normal growth of albino rats. Chinese velvet bean protein prepared by dialysis, however, whether or not cystine was added, resulted in lack of

[6] The protein was isolated from a different sample of beans than that which was used in the feeding experiments. The beans used in this investigation were furnished by the Bureau of Plant Industry of the United States Department of Agriculture.

growth. The bean meal, on the other hand, cooked or autoclaved and supplemented with either cystine or casein, resulted in nutritional failure, manifesting itself in lack of growth, regurgitation, diarrhea and ultimately death.

2. The relative probability of protein toxicity, associated toxic substance (dihydroxyphenylalanine ?), or partial indigestibility of the protein, as causes of the failure of the dialyzed preparation, are discussed. An associated toxic substance seems the most probable source of the nutritional disturbances observed in the case of the steam-cooked meal.

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THE MECHANISM OF THE RECOVERY OR MAINTENANCE OF SYSTEMIC BLOOD PRESSURE AFTER COMPLETE TRANSECTION OF THE SPINAL CORD[1]

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Physiologists have been divided in opinion for years as to the criteria to be observed in arriving at an estimate of the function of particular levels or regions of the central nervous system. The methods of experimentation most commonly employed involve anatomical destruction of some part, or anatomical solution of continuity of conduction pathways in the central system. Two stages may be observed in the course of the recovery of an animal from an experimental lesion of any portion of the nervous system; there is first the acute state, during which the effects of the lesion are most severe or most noticeable. Following this, there is a period in which the effects grow generally less severe or less noticeable until a state is reached at which no further change occurs. The effects observed in the first state, but which disappear in the course of the second or recovery period, are commonly attributed to shock. The progress during the second period is often attributed to the general failure of the shock effect. The ultimate state of recovery, in which there is the deficiency of function, is taken as the truest index of the function of the injured portion of the nervous system. But there is no general agreement even here, for some insist that if shock could be completely eliminated, the recovery would be still more complete, while others feel inclined to set a limit to the effects of shock, and consider that the minimal deficiencies of function indicate the complete subsi-

[1] The author gratefully acknowledges her indebtedness to Prof. W. T. Porter for the generous hospitality of his laboratory in which these experiments were done, and to Prof. F. H. Pike for his guidance and help throughout the work. The paper was submitted as a thesis for the degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University.

dence of all shock effects, and are therefore to be taken as the true criteria of the function of the injured portions of the nervous system.

There is in the literature no consistent statement of the functional organization of the nervous system. According to the importance one attributes to such vague and generally unmeasured influences as shock, depression, loss of tonus and other like terms for unknown processes, one may take either of two positions and ally oneself with either of two general hypotheses of functional organization. Briefly stated, these two positions are:

1. The segmental hypothesis of Goltz and his followers, in which shock plays an all important rôle. In its general form, the hypothesis states that the same level or division of the nervous system has essentially the same function in all types of animals. The reason that a dog does not recover as completely as a frog after decerebration is that shock is more severe and more persistent in the dog. No limit is assigned to the effects of shock. One important consequence of this hypothetical omnipotence of shock is that there is, according to this hypothesis, no localization of motor function in the cerebrum.

2. The theory or hypothesis of cerebral localization. The most extensive statement of this theory at the present time is due to von Monakow. He clearly recognizes that one must assign some limits to the effects of shock, and takes the position that all the effects of a lesion in the central nervous system of a mammal—for example, a dog—are not due to shock. He considers that the shock effect ultimately recedes completely, and that the final deficiencies of function observed after a considerable period has elapsed are to be taken as an index of the function of the lost or injured portions of the nervous system. An important conclusion is that there has been a shifting of function in the phylogenetic development of vertebrates, and that we have phylogenetically new, as well as phylogenetically old, sensory and motor pathways for somatic functions.

A modification of this second position consists in limiting still further the effects of shock, and of introducing the idea, due to a suggestion of Hughlings Jackson, that there may be a quantitative change in the amount of nervous energy passing over the remaining pathways, or through the remaining levels, of the nervous system after an injury to one or more of them. It is obvious that a quantitative change, tending to an increase in the functional capacity of the remaining levels or pathways, might tend to decrease the final deficiency of function, and so lead to too low an estimate of the actual work of the injured portion. A fuller

discussion of these views is given by Pike (1). It may be remarked in passing that the question of shock is bound up with the question of the validity of our methods of investigation of the nervous system, and the soundness of our views on its function.

The difficulty in arriving at an estimate of the actual importance of shock, or of the effect of a quantitative change in the function of the remaining pathways after an experimental lesion, has arisen from the fact that it has been difficult, or generally impossible, to get at any independent quantitative determination of either factor in the equation. Until this is done, the solution of a single equation of two or more unknown quantities must remain indeterminate.

It is desirable to find some physiological system whose functional continuity may not be completely interrupted by a total transverse lesion of the spinal cord, and then to determine, after an interval of recovery, the effect of temporary or permanent elimination of the parts lying above or below the level of transection. If, then in investigating the functional organization of the nervous system by noting the results of lesions, it were possible to study the relationships in some system in which the outflow from the central system is widely distributed, one should be able, by isolating different segments of the outflow, to derive some information as to the functional significance of these different levels of the central mechanism relative to the system under consideration.

The vascular system fulfils these conditions admirably. It is generally agreed that a vasomotor center lies in the medulla. Efferent vasomotor fibers leave the spinal cord with the first or second thoracic roots through to the thirteenth thoracic root, the heaviest outflow, according to Langley, occurring in the region of the sixth thoracic root. No vasomotor fibers leave the cord in the cervical region and few or none in the lumbar and sacral regions.

In view of the fortunate anatomical arrangement of the efferent portion of the vasomotor system, it seemed feasible to effect an actual separation of the higher centers from the spinal cells by complete transection of the spinal cord.

In the normal life of the individual the blood pressure is maintained at a practically constant level through the cardiac nerves and the vasomotor mechanism. It is common knowledge that the immediate result of complete transection of the spinal cord (in the upper thoracic region) is a severe fall of blood pressure and a cessation of skeletal reflexes involving muscles whose afferent and efferent nerves enter or leave the spinal cord below the level of transection. The blood pressure does not return

to its normal level and only a part of the somatic reflexes return during the course of an acute experiment. In general the higher the level of transection of the thoracic cord, the greater the fall of the blood pressure. The severity of the effect is independent of the method of blocking conduction in the cord (2). If, however, the animal be kept alive for a period of days, there is a more extensive recovery of the skeletal reflexes and the blood pressure is said to approach the level maintained before the injury. The general statements in the literature are, however, too vague to permit of drawing any sound conclusions. More exact control data have recently been gathered which serve as a basis of comparison for the results given in this paper.

To what, we may ask, is this recovery due? If we cling to the segmental theory, we might advance the hypothesis, as Goltz did, that the recovery of blood pressure is due to the subsidence of the effects of shock which had depressed the spinal cells of origin of efferent vasomotor fibers lying below the level of transection. If, however, we believe that, in the development of the higher animal forms there has been a greater tendency toward cerebral localization, we might look to the center in the medulla oblongata and the outflow still in functional connection with this bulbar center for an explanation of the return of blood pressure to or maintenance at the higher level.

After allowing a period for recovery, one should be able to determine: *a*, whether or not there is a return of blood pressure; *b*, if so, to what extent recovery has taken place; and *c*, whether the mechanism on which the recovery of blood pressure depends, involves the medulla oblongata and the efferent vasomotor fibers above the level of transection, or the spinal cells below the level of transection from which efferent vasomotor fibers take their origin.

These three points comprise the objective of a series of experiments described in this paper. It was believed that by measuring the blood pressure at varying intervals after transection of the cord, the presence or absence of recovery and its extent should be shown; by functional elimination of the medulla oblongata through occlusion of the head arteries, specific information should be gained regarding the rôle of the medulla in maintaining this pressure, since it is difficult to see how any operation on the medulla can affect the isolated segment of the spinal cord. Measurements of the blood pressure obtained after cerebral anemia, the spinal cord being anatomically intact, served as a control for the general level of spinal pressure. If the spinal cells were responsible for the recovery of the blood pressure then the functional elimi-

nation of the medulla ought not to affect the level of blood pressure seriously. If, on the other hand, the recovery of blood pressure had occurred through the center in the medulla and the spinal cells were normally dependent on the higher center for their functional activity, elimination of the medulla should result in a considerable fall of blood pressure.

Before presenting the results obtained by the experimental procedures outlined above, a brief consideration of the literature which bears most directly on these points is advisable.

Goltz (3) transected the spinal cord of dogs between the thoracic and lumbar segments. Other reflexes than those involving the vasomotor mechanism were the object of the investigation and the observations on the effect of the lesion on vasomotor responses appear to be incidental. No actual measurement of blood pressure is recorded. Goltz noticed that after transection the hind feet became warmer, due to vasodilatation, and that gradually the difference in temperature of the fore and hind feet disappeared, and later the hind feet were even colder than the forefeet. Comparing these results with those previously obtained on frogs, he concluded that the lumbar cord was the vasomotor center for the hind limbs. Goltz assumed that the temporary abolition of reflexes after section of the cord was due to the suppression or inhibition of the normal function of the cells of the lower level of the cord by shock. What recovery of function occurred was due to the passing of the effects of shock, and failure to recover completely was attributed to the persistence of shock.

Twenty odd years later Goltz (4) made a series of experiments on dogs in which he removed a large part of the spinal cord, in successive operations. The upper level of transection, he states, was in some cases as high as the fifth or sixth cervical vertebra; it was less dangerous, however, and a more complete recovery occurred if the first transection was made in the region of the third thoracic segment. These animals lived for months apparently in good health, from which Goltz drew the conclusion that it was erroneous to assume that the spinal cord of warm-blooded animals was necessary for the carrying on of the processes of nutrition, for the regulation of the size of the blood vessels and the maintenance of body heat.

One might reasonably ask, then, what has become of the vasomotor center which had previously resided in the lumbar cord? Sherrington (5) states that complete transection of the spinal cord of the dog through the eighth cervical segment is followed by a severe fall in the general

arterial blood pressure. After some weeks the blood pressure, with the animals in the horizontal position, will often be found practically normal. The arterial pressure in one dog, taken 300 days after transection, was 90 mm. Hg. The blood pressure found is maintained by the spinal cord, and not by peripheral mechanisms, as is shown by the fact that a profound fall occurs on destruction of the cord in the thoracic region. One should, therefore, exercise some care in comparing the results of Goltz' experiments on destruction of the spinal cord where the transection is made at the level of the second or third thoracic segment, and a similar destruction where the transection is made at the level of the eighth cervical segment. The experiments reported here are related more closely to Goltz's findings than to the findings of Sherrington.

Langley (6) further investigated the existence of spinal vasomotor centers with the use of strychnine. The cats were decerebrated and the spinal cord transected in the mid cervical region. By this procedure all question of the possible influence of higher centers on blood pressure was eliminated since the level of transection was above the outflow of vasomotor fibers. Any change in blood pressure then would necessarily be attributed to the activity of the cells of origin situated in the spinal cord. On the injection of a small amount of strychnine a prompt and extensive rise of blood pressure was obtained. The conclusion was drawn that the strychnine had stimulated spinal vasomotor centers. Langley confirms Pike's (7) observation that the intravenous injection of curare is followed by a further fall in the blood pressure of the spinal animal. He considers this fall to result from a decrease of muscle tone. The justification for considering these spinal cells of origin of efferent vasomotor fibers as vasomotor centers is not clear. It is not to be denied that these cells may be stimulated in different ways to give vascular responses, but the real question is, what is the source of these impulses in the natural life of the animal? Do they come from higher centers or do the spinal cells possess an actual autonomy? Electrical stimulation of the cord, as well as strychnine, causes marked vasoconstriction, but neither fact offers sufficient ground for assigning independent vasomotor centers to the spinal cord.

If, as Langley states, fibers coming in through the dorsal roots make connection with the vasomotor cells in the spinal cord, the visceral sensory and motor system has an arrangement similar to that obtaining in the somatic sensory and motor neurones of the spinal cord. Such a similarity might be expected on general grounds, but the question of morphological relationship still remains to be answered. Some investi-

gators believe that the somatic sensory fibers send collaterals directly to the ventral horn cells. Such a connection in the case of the sympathetic system must be effected between the afferent fibers and the preganglionic cells in the cord. If the post-ganglionic cells of the sympathetic system correspond to the ventral horn cells of the somatic motor system, the regions of arborization between the afferent fibers and the motor cells may not lie at the same place in the two arcs—somatic and visceral.

On the other hand, there is striking evidence in favor of the localization of the vasomotor center in the medulla. Ranson (8) sectioned only the apices of the dorsal gray columns at the level of the first thoracic roots. In this way certain afferent vasomotor fibers alone were interrupted, efferent channels for vasomotor or skeletal reflexes being unaffected. Thus all possibility of what Von Monakow calls the diaschisis effect was ruled out, since diaschisis is held to produce its effect over efferent pathways alone. Vasomotor reflexes were tested by stimulation of the brachial and sciatic nerves. As a result of such a lesion the usual rise of blood pressure obtained on stimulation of the sciatic nerve was abolished or markedly decreased whereas the rise obtained from stimulation of the brachial nerve persisted in undiminished intensity. The interpretation placed on these results was that the pressor pathway from the lower level had been interrupted before reaching the higher centers, whereas the central connections of the pressor fibers entering the cord with the brachial nerve above the lesion were intact. Thus Ranson accounts for the difference in response of the two nerves which, in an uninjured animal, produce equal effects on stimulation.

THE EXPERIMENTAL METHODS, MATERIAL AND RESULTS

The material used and the preliminary operation. Half-grown cats, preferably females, were selected for operation when available. As a matter of fact, adult cats of both sexes were used at times. Ether was the anesthetic employed. The fur was clipped over the field of operation with scissors. The field was then scrubbed thoroughly with a solution of mercury bichloride 1:1000. Towels which had been boiled and wrung out of bichloride covered the surrounding parts. Instruments were sterilized by boiling.

A longitudinal incision was made in the median dorsal line in the cervical or thoracic region, depending on the level at which the transection was to be made. The muscles were cut from their attachments to the spinous processes, held back by means of a retractor and the vertebrae

scraped free of muscle. Usually the spinous processes and arches of two vertebrae were removed with bone forceps. Care was taken not to extend the removal of the arch of the vertebra more than 2 or 3 mm. from the median line lest the hemorrhage be too severe. The wound was thoroughly washed out with hot sterile water and dried with sterile cotton. The following method of cutting the spinal cord was found to be most successful. A small pair of fine curved forceps was cautiously placed under the cord, which was then gently lifted partially out of the canal and a quick transverse section made with scissors. This procedure involved a slight hemorrhage from the anterior spinal vessels, but the bleeding soon subsided. The wound was then flushed clean with hot sterile water and closed with sutures. Sterile catgut or cotton thread was used. The wound was covered with sterile gauze and cotton and then bandaged. In no case did suppuration or meningitis occur.

Twenty-eight rabbits and one cat were used in the earlier experiments, but in no case was a completely successful measurement of blood pressure obtained. (A measurement without any operation affecting the medulla oblongata was made on one rabbit.) Nineteen cats were used in the final experiments, and measurements of blood pressure, partially or wholly successful, were made on twelve of them. Three other preliminary observations made by Mrs. Winkin, to be published by her, gave results in entire accord with mine.

The post-operative care and condition of the animals. Upon this hung the success or failure of the experiment. Snuffles, anthrax and pneumonia brought several to an untimely end, but these maladies could be attributed to pre-operative infection and not to any untoward result of the transection.

The immediate result of the transection was a severe drop in body temperature, which the animal was unable to bring back to normal by its own heat-regulating mechanism. It was placed on a bed of hay in a warm pan. An electric pad supported by the sides of the pan covered the cat and supplied the necessary heat. In one case the current was inadvertently turned off from the pad on the first night after the operation. In the morning the cat was very cold and weak. Heat was at once supplied, with favorable results, but after the failure of the heat on the second night the animal died. After 24 to 48 hours, depending on the condition of the cat, it was removed to the recovery cage. After the first 48 hours the heat-regulating mechanism appeared to regain its function in large part.

The heat-regulating mechanism of the rabbits, however, showed little tendency to recover its former efficiency. A severe fall of body temperature, ranging from 4 to 10°F., followed immediately upon transection of the cord. Furthermore, it was necessary in many cases to resort to the almost constant use of the electric pad in order to keep the temperature of the animals even approximately normal. The fluctuations of temperature were less marked after hemisection than after complete transection. This is shown by a comparison of the temperature variations in rabbit 10 during the period November 15 to November 25 and November 26 to December 1 and by a further comparison of the variations in rabbit 14. The results are tabulated below:

NUMBER OF EXPERIMENT	LOWER AND UPPER LIMITS OF BODY TEMPERATURE	
	After hemisection	After transection
10	96.4°–101.8°	92.2°–103.2°
14		92.8°–107.2°

The greater recovery of the heat-regulating mechanism in the cats may have been due largely to the return of muscle tone and the increased muscular activity, to be discussed in a subsequent paragraph. The lack of recovery in the rabbits, on the other hand, may bear some relation to the fact that the rabbits were comparatively inactive.

The cats were fed once a day, the food consisting of meat, fish heads, liver, and bread and water in which meat had been boiled. Appetites were good on the whole and in some cases the animals were greedy.

The bladder was emptied once a day by manual pressure. Considerable pressure was required for the first 2 or 3 days, particularly in male cats, but after that the urine could be expressed fairly easily. After 16 days or more there was a certain amount of spontaneous voiding of the urine. Complete spontaneous evacuation was not usual. In one cat which was pregnant, partial recovery of the automaticity of the bladder was noticed on the 4th day. After the 8th day the recovery was practically complete. Defecation occurred normally in all the animals.

The cats were rather active, some of them very active indeed. After the 1st to 3rd day, the muscles innervated from the isolated part of the spinal cord regained their tone in part. The flexors of the hind legs could be reflexly stimulated by pinching the toes, and a crossed extension reflex was produced by tapping the knee. Also on holding a cat that had survived the transection 16 days by the forelegs so that the hind legs hung free, the progression reflex of the hind legs was obtained. Cat

18, in which the transection was at the level of the seventh cervical segment, was unable to support itself on its forelegs.

In cat 14, which lived for 32 days after the transection of the spinal cord, the impact of the hind legs against the side of the cage as the animal dragged itself about by its fore limbs was often sufficient to produce strong extension of both hind limbs, upon which there was superposed a tremor with a frequency of about four or five in the second. This rarely lasted for more than a second.

Only two of the cats were afflicted with sores. Goltz remarked that these sores, occurring on the part of the body below the level of transection, were difficult to avoid and extremely difficult to heal if they appeared. In one case the sore appeared after 10 or 12 days, and in the other after 28 days. Both were on the thigh. It is probable that the sores came as a result of rubbing against the wire netting of the cage. This netting was covered with a thick bed of hay, but both of these cats were extraordinarily active and the netting was uncovered in places as they crawled about the floor. Furthermore, as the recovery of the mechanism for emptying the bladder progressed, it became impossible to keep the animals dry, and this acted as a further and continuous source of irritation.

Three cats, 3, 16 and 20, in which the level of transection was high, evinced sudden and grave signs of collapse, which proved fatal in one case. First signs of difficulty were observed, in the case of cats 3 and 16, when they were placed on their backs for the purpose of expressing the urine. Their heads were held down with extreme gentleness. In spite of this they became greatly agitated and began pawing violently with the fore legs and struggled to free themselves. Close upon the heels of this unusual exertion followed slow and labored breathing and even convulsive movements, as the respiration became more infrequent and gasping. The thoracic wall was compressed rhythmically in an effort to restore normal respiration. The efforts were attended by success in the case of cat 16 but cat 3 continued to manifest such serious dyspnoea that it was necessary to resort to artificial respiration. At this time the pupils were widely dilated and the corneal reflex absent. Under the more effective measure recovery was brought about, but rather than risk another collpase preparations were made to record the blood pressure at once. The protocol of the experiment follows.

Cat 3. Blood Pressure Tracing. February 20, 1920. A little ether—Tracheotomy—Heart slow and respiration bad.

8:05 p.m. Left vagus cut: Mean blood pressure 50 mm.

8:08 p.m. Right vagus cut. Corneal reflex present: No change. Momentary fall.
8:14 p.m. Ligation of cerebral arteries: Rise of 7 or 8 mm., reaching 55 mm.
8:18 p.m. Release of cerebral arteries: Pressure had fallen to 40 mm.
8:25 p.m. Abdominal aorta compressed: Rise from 40 to 130 mm. (N.B. good condition of heart.)
8:40 p.m. Stimulation of central end left vagus: no effect.
8:43 p.m. Stimulation of left sciatic: Fall of 5 mm., then slight rise to 50 mm. After stimulation stopped pressure fell to 44 mm.
8:45 p.m. Stimulation of central end left vagus: No effect.
8:47 p.m. Ligation of cerebral arteries (this ligation was apparently incomplete as respiration was not interrupted): Rise from 44 to 58 mm. returning to 44 mm. as final result of occlusion.
8:55 p.m. Readjustment of ligatures making ligation more complete: Rise from 40 to 48 mm.
9:00 p.m. Stimulation of sciatic: Rise from 44 to 50 mm.
9:05 p.m. Pressure gradually decreased to level of 34 mm.
9:09 p.m. Asphyxia: Small rise 5 mm. then decline to 0 (base line).

The above protocol shows well the condition of the cat. Just why it should have been in such a precarious state that the mere placing of it on its back was followed by the train of distressing symptoms cited above, is difficult to say. Apparently, compensatory mechanisms for maintaining blood pressure in emergencies were not operative, so that relatively slight exertion produced profound changes in internal conditions. Considerable though not complete anemia of the medulla probably occurred very soon after the cat began to struggle. It can be seen from the very slight rise in blood pressure on ligation of the cerebral arteries at 8:14 that function of the medulla had already ceased. Furthermore, it is clear that function was not restored by the higher level of pressure obtained on compression of the abdominal aorta at 8:25 since the subsequent ligation of the cerebral arteries at 8:47 was followed by no significant rise.

The case of cat 20 was somewhat different. Transection of the cord had been completed, the operation being attended by little hemorrhage. As the wound was being closed the respiration appeared to become somewhat irregular. The corneal reflex was present, as no ether had been given for some minutes. A few minutes later, however, the pupils were found to be widely dilated and the corneal reflex gone. At this time it was noticed that the cat was lying on the operating board in such a way that the abdomen sagged in consequence of lack of support. Respiration had failed. The cat was placed on its back and the thoracic walls compressed rhythmically. The impulse of the heart against the chest

wall showed the beat to be slow and weak. Warm saline was quickly injected into the stomach. There was no improvement in the condition. Tracheotomy was done, the thorax opened and artificial respiration was begun. The heart was flabby and beating weakly. The femoral vein was exposed and about 25 cc. of warm saline injected. The condition of the heart improved, the tone of the ventricles increasing and their action becoming more vigorous. The improvement was only temporary. A few drops of adrenalin were then tried. Again the heart was bolstered up for a few minutes only. Despite all remedial measures, there was no indication of real recovery.

The condition of the cardiovascular mechanism after an interval of recovery. After an interval varying from 2 to 32 days, the extent of recovery of systemic blood pressure was determined. The usual method of procedure was ether anesthesia; tracheotomy; preparation for occlusion of the head arteries and preparation for the stimulation of the brachial and the sciatic nerves and the spinal cord below the level of transection. Anemia of the brain was produced at will, by tightening ligatures placed around the left subclavian artery below the origin of the vertebral and around the right subclavian and carotid at their branching from the innominate (9). The cannula for blood pressure was placed in the left carotid artery in all but one instance and, in that case, the left carotid was also ligated. Artificial respiration was employed whenever it was necessary. It was always maintained during the period of occlusion of the head arteries. Twelve observations were made, including one cat with cord intact. Blood pressure was measured with a mercury manometer. Throughout the paper, the rise of blood pressure following occlusion of the head arteries is called the anemic rise, to distinguish it from the asphyxial rise tested out at the close of the experiment.

Amount of recovery of systemic pressure. The amount of recovery of blood pressure seemed to be influenced largely by the level of transection and, to some extent, by the length of time elapsing after the operation. Thus the average levels of blood pressure were 38 mm., 90 mm., 102 mm. and 114 mm. in cats transected at the level of the seventh cervical, first thoracic, third thoracic and fifth thoracic respectively. The periods of recovery in these cases were 6 days, 3 days, 10 and 32 respectively.

In the one rabbit in which the blood pressure was measured, the mean level was found to be 60 mm. Hg. No reflex vasomotor response was obtained on stimulation of the brachial or sciatic nerves. Slight variations in the level of blood pressure were produced by the stimulation of various nerves in the cervical region. No occlusion of the head arteries

or other means of analyzing the mechanism of the maintenance of this pressure was tried. The level of the transection of the spinal cord was in the region of the third thoracic segment.

The anemic rise of blood pressure and the pressure maintained by the isolated portion of the spinal cord. When a fairly constant level of blood pressure had been reached after the animal was anesthetized, anemia of the brain was produced by occlusion of the cerebral arteries. The immediate result of this procedure was a sharp rise—the anemic rise—which was usually interrupted by a temporary fall due to the inhibitory action of the vagus. The anemic effect again predominated, as shown by the continued rise in pressure. The pressure at the peak varied from 54 to 154 mm. A gradual fall then ensued and the constant level reached subsequent to this fall was taken as the blood pressure maintained by the spinal cord. The blood pressure curve is shown in figure 1.

The average spinal pressure obtained in these cats clearly indicated two things.

First: That the pressure obtained could scarcely be attributed to shock, since a comparison with cats with the spinal cord anatomically intact but with the medulla eliminated by anemia, or with cats in which transection of the cord had been done a few minutes before the measurement of blood pressure, shows that the pressures are nearly the same under all the various sets of conditions, or that the pressure is even lower in cats which have survived the transection several days. Judging by the usual criteria, one would expect the partial or complete subsidence of shock effects with the passing of time. Thus the average spinal pressure in the cats cited above was 26, 52, 48 and 35 mm. respectively and the period of recovery as stated before, was 6, 3, 10 and 32 days. The spinal pressure obtained in the cat with cord anatomically intact was 56 mm. This measurement is in line with results obtained by Stewart (10) and Pike (7), who found the average spinal pressure in a series of 60 or more normal cats to lie between 50 and 60 mm. Hg.

Second: That the isolated portion of the cord did not acquire any significantly greater influence in the recovery of the blood pressure and its maintenance with the passing of time up to the limit of life in this series of experiments. Thus a cat whose spinal cord was transected at the level of the first thoracic root showed a spinal pressure of 52 mm. after 3 days; another cat with the spinal cord transected at the level of the third thoracic segment had a spinal pressure of 40 mm. after 18 days; in a third cat in which the transection was made at the fifth thoracic root, a spinal pressure of 35 mm. was obtained after 32 days.

The effects of stimulation of the peripheral part of the spinal cord and the splanchnic nerves. It may be suggested that the low level of pressure maintained by the isolated portion of the cord, lower even than that found in normal cats upon occlusion of the cerebral arteries, was due to "inhibition" of the spinal cells of origin of efferent vasomotor fibers

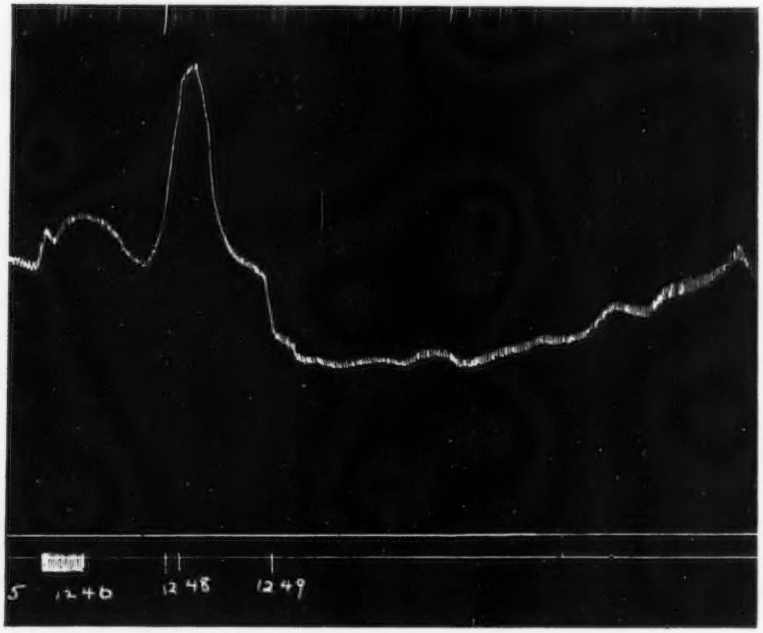

Fig. 1. Blood pressure curve of cat 13, showing blood pressure maintained before and after occlusion of the head arteries and the anemic rise. Head arteries occluded at 12:48 and released at 12:49. Transected at level of 1st thoracic 3 days before blood pressure was measured. The pressure rose after release of the head arteries until it reached the pre-occlusion level.

from the shock of transection. In order to test the validity of such an argument, the spinal cord was stimulated electrically just below the level of transection. An immediate and pronounced rise of blood pressure followed. That this electrical stimulus was greater than any natural stimulus, and for that reason might be able to pass the mysterious block imposed by shock, is a position not wholly tenable in view of the

fact that the mere placing of the electrodes on the cord was sufficient to produce a marked rise.

The muscular response which followed the stimulation of the cord was usually pronounced. There was extension of the hind limbs, spreading of the claws and, in one case, the tail was elevated and wagged violently. A rise of blood pressure was also obtained in the two cases in which branches of the right splanchnic nerve were stimulated.

Adequacy of the heart. It may be suggested that the consistently low blood pressure which was maintained in each case by the isolated portion of the spinal cord alone was due rather to an inadequate heart action than to the failure of the central vasomotor mechanism in this portion of the cord. That the low spinal pressure could not be laid to a weak heart action was shown by the great rise of pressure (12 mm. to 78 mm.) obtained on clamping the abdominal aorta. By constricting the aorta the peripheral resistance was considerably increased. If the heart were weak, even a great increase in peripheral resistance would not be effective in producing a marked rise of pressure. On the other hand, a strong heart alone cannot raise, or even maintain, the blood pressure at a normal level against the depressing effect of the widely dilated splanchnic vessels.

Effect of section of the nervi accelerantes or removal of the stellate ganglia. An estimation of the rôle of the nervi accelerantes in bringing about the rise of blood pressure resulting from anemia of the medulla oblongata could be reached only by a comparison of the anemic rise obtained when the extrinsic cardiac nervous mechanism was intact, with the anemic rise obtained after the section of the nervi accelerantes or the removal of the stellate ganglia. The difficulty of getting a recovery of the function of the medulla oblongata after the first occlusion in cats which had undergone complete transection of the cord, seriously hampered the acquisition of additional experimental data which it was hoped to gain for a further analysis of the mechanism of the anemic rise of blood pressure after transection of the spinal cord. First, the effect of occlusion of the head arteries with the extrinsic cardiac nerves intact was determined. In some cases however the effect of the vagus was so marked during the period of cerebral anemia that double vagotomy was advisable. In a few experiments it was possible to try a second anemic rise by ligation of the head arteries after section of the nervi accelerantes or excision of the stellate ganglia. It may be remarked in passing that in cats with spinal cord intact, several occlusions and recoveries are possible in the same animal.

It seemed likely that after transection of the spinal cord, the extrinsic cardiac nervous mechanism might be partly responsible for the anemic rise which follows occlusion of the head arteries. In three cats which showed an anemic rise of 74, 58 and 38 mm. with the stellates intact, a later occlusion of the head arteries resulted in only slight elevations of pressure—10, 6 and 6 mm. respectively. Where the spinal cord was intact, removal of the stellates had little effect on the rise of blood pressure. Occlusion of the head arteries was followed by a rise of 70 mm. in this case when the stellates were uninjured and by a rise of 140 mm. after their removal. The pre-occlusion level was lower after removal of

TABLE 1

Showing the length of life of the animals and the blood pressure after spinal transection

NUMBER OF EXPERIMENT	LEVEL OF TRANSECTION	DAYS ANIMAL LIVED	AVERAGE SYSTEMIC BLOOD PRESSURE	AVERAGE SPINAL BLOOD PRESSURE	ANEMIC RISE ON OCCLUSION OF HEAD ARTERIES	
					Stellates intact	Stellates removed
			mm.	*mm.*	*mm.*	*mm.*
7a	Cord intact	0	120	56	70.0	140
6	VII C	5	62	38	74.0	10
18	VII C	6	38	26	6.0	
13	I T	3	90	52	58.0	6
3	II T	6	50	40	7, 8	
19	II T	2	56	34	10, 8	
4	III T	10	102	48	36	
11	III T	18	76, 90	40	38	6
15	III T	11	38	40	12	
2	V T	6				
5	V T	12	90	34	64	
14	V T	32	114	35		

the stellates than before, but the ultimate level of blood pressure after occlusion in these two instances was practically the same, 170 and 180 mm. respectively. Thus it seems that when all efferent vasomotor routes are open, as in the intact spinal cord, the reaction of the medulla against increasing anemia is shown by the sending out of vasoconstrictor impulses along the cord. Even when the cord is transected, the channels left open above the lesion are effective in compensating temporarily for the loss of blood to the medulla, but when, in addition to the transection, the stellates are removed, it is not possible to obtain so great a compensatory rise of pressure.

The general condition of the cardiovascular system as indicated by the measurement of blood pressure is shown in table 1.

Effect of stimulation of afferent nerves. It would be expected that stimulation of nerves such as the brachial and sciatic, would produce results in the cat with transected spinal cord different from those obtained in the normal cat. The level of transection might determine the extent of the response which the brachial would give on stimulation, i.e., if the level of transection were low enough to permit of a considerable efferent vasomotor outflow over the upper roots of the thoracic portion of the cord, the effect would be greater than if the transection were sufficiently high to interfere with this outflow.

In the case of the sciatic, one would expect a failure of its usual effect,—provided its afferent fibers actually do make a functional connection in the spinal cord with the cells of origin of the efferent vasomotor fibers—only when the level of transection passes so low in the cord as to fall below the segments from which these efferent fibers arise. But if the afferent fibers of the sciatic make their usual functional connection with the efferent fibers through the medulla oblongata, the transection of the cord should greatly decrease its usual effect on blood pressure. The stimulation of afferent nerves while the function of the medulla was intact and after its paralysis by anemia might be expected to yield some results of interest in relation to the rôle of the medulla oblongata and the spinal cord in the maintenance of blood pressure after spinal transection.

Stimulation of the brachial plexus and the sciatic nerve gave the following results before and after occlusion of the head arteries:

NUMBER OF EXPERIMENT	LEVEL OF TRANSECTION	BRACHIAL		SCIATIC	
		Before occlusion	After occlusion	Before occlusion	After occlusion
		mm.	*mm.*	*mm.*	*mm.*
18	VII C	2	0	−4	0
11	III T	20	1	10	2
15	III T	6	6	2	6
7a	Cord intact			28	0

No. 15, which gave such different results from no. 11, was in labor at the time the blood pressure was measured. Stimulation of the sciatic produced no significant rise before occlusion of the head arteries except in no. 7a. After occlusion, or failure of the medulla for some other reason, no significant rise occured except in one case in which an increase of 12 mm. was obtained. A rise of 8 mm. followed stimulation of the sciatic when the medulla oblongata of this animal was functional.

Effect of stimulation of the vagus. The effects of stimulation of the central end of the vagus deserve separate treatment because of the known anatomical nuclei of origin of its fibers, and its undoubted functional connections with the medulla oblongata. The result of stimulation of the vagus depended largely upon the state of the other vagus. When one vagus was intact, stimulation of the central end of the cut vagus when the medulla was functionally active brought about a considerable reflex fall, varying from 14 to 42 mm. In one case no reflex effect whatever was obtained, although stimulation of the intact vagus produced a great slowing of the heart and a marked fall of blood pressure. When both vagi were cut, stimulation of the central end of one usually had no effect on the blood pressure if the level of transection was high. In one case, however, a fall of 32 mm. was obtained under these conditions. All reflex responses of the vagus failed after paralysis of the medulla by occlusion of the head arteries.

In one cat a separate depressor nerve was found. Stimulation of the central end produced a marked fall of blood pressure.

Effect of asphyxia. It was found that on cessation of artifical respiration at the close of the experiment, and after complete functional elimination of the medulla, an asphyxial rise of blood pressure occurred in some animals. This rise was not always great, sometimes only 4 or 5 mm., though at other times it was as much as 14 or 18 mm. Usually this rise was followed by a gradual fall to base line as the heart became slower and weaker and finally stopped altogether. In two cases the initial rise and subsequent fall were followed by a second rise, one of 14 mm., another of 46 mm. The asphyxial rise was not greater in cats which had lived a considerable time after spinal transection than in cats which had lived only 2 or 3 days.

Pilomotor activity, frequently seen in asphyxiation, was observed in only one of the present series of animals (cat 19). Two minutes after the artificial respiration was stopped, the hind limbs and tail began to twitch. In a few moments more the hairs on the back and tail began to stand up.

Another effect of asphyxia is seen in the spontaneous evacuation of the bladder. This was noticed in two cats, 6 and 15, micturition occurring 9 minutes after the cessation of artificial respiration in one and 3 minutes after the beginning of asphyxia in the other. It is of interest, and possibly of some importance, that the second cat was pregnant and that the bladder had already acquired considerable automaticity during the interval of recovery. Spontaneous micturition has been observed by Pike,

during the asphyxial period, in cats with uninjured spinal cord, but with medulla paralyzed by anemia several hours previously. He also cites an instance of spontaneous emptying of the bladder, with active contraction of its muscular fibers, as much as 1 hour after the death of the cat. The abdominal wall had been opened soon after death, and the actual change in the size of the bladder was observed.

The duration of the heart beat after artificial respiration had been discontinued was found to be somewhat longer after the transection of the spinal cord than in cats with the cord intact. Thus in two normal cats the heart action continued 8 and 9 minutes after the beginning of the asphyxial period, whereas, in ten cats in which the spinal cord had been transected, the heart continued to beat from 8 to more than 23 minutes, the average being 12 to 15 minutes. There was no relation apparently between the level of transection and the duration of the heart beat in asphyxia.

Some incidental observations made during the course of the experiments. Certain incidental observations of interest in connection with the general functional condition of the spinal cord were made in addition to the results which were more directly connected with the main problem. The recovery of the heat-regulating mechanism, of the reflexes of the skeletal muscles, and the mechanism for micturition have already been discussed under the section on post-operative care.

Onset of rigor mortis. Upon arrival at the laboratory one morning at 8:30 o'clock, one cat was found dead from accidental strangulation. The body was already cold. However, it seemed worth while to try resuscitation, and within 3 minutes tracheotomy had been done and artificial respiration begun. The thorax was opened, the abdominal aorta clamped, and massage of the heart started. This was continued for about 40 minutes, but the only evidences of returning life were a few feeble flickers of the ventricles, so feeble indeed that at 9:15 first aid measures were abandoned and reluctantly the animal was pronounced beyond hope. At this time rigor mortis of the fore limbs was observed, though there was no post-mortem rigidity of the hind limbs. The condition of the fore limbs had not been noticed at the time tracheotomy was done. Five hours later rigor mortis was observed to have set in in the hind limbs. No other observations were made in the interval.

Ineffectual labor. One cat was in advanced pregnancy at the time of the operation. A good recovery was made. On the 10th day following the transection signs of labor were evident. There was considerable activity on the part of the foetuses. The old cat became quiet and

"dopey." The progress of labor was watched for 5 or 6 hours, but delivery did not occur. On the morning of the 11th day one fetus in breach presentation was found only partially delivered. Examination showed it to be dead. The other embryos were still alive and their movements in utero could be observed through the maternal body wall. Since it was only a matter of hours before septic processes would start in the mother cat, the blood pressure was measured at once.

The effect of intravenous injection of fluids. In a few cases where the blood pressure was very low, an effort was made to raise it by injecting warm saline into the femoral vein before ligating the head arteries. The saline was injected with a hypodermic syringe, each injection of 5 cc. being followed by a rise of 4 or 5 mm. The heart action was also greatly improved. This rise was not maintained, however. On one occasion a few drops of adrenalin were given with the saline. The customary sharp rise ensued but this too was transitory. The immediate response which the injection of even small quantities of saline produces in cats with transected cord is in direct contrast to the reaction of those where spinal cords are intact. In the latter the level of blood pressure is not appreciably affected until large quantities of fluid have been injected. The reaction to the intravenous injection of fluids in some animals whose spinal cord was transected but whose medulla oblongata was intact, was essentially that of a purely spinal animal. The medulla was exerting little or no influence on the blood pressure.

Prominence of the nictitating membrane. In cats in which the level of transection was high—seventh cervical or first thoracic segment—the nictitating membrane was more than usually prominent, being drawn well toward the pupil. This is probably due to injury to the fibers of the sympathetic system which emerge from the spinal cord in the upper thoracic segment, pass through the stellate ganglion, and up to the eye.

THE GENERAL THEORETICAL SIGNIFICANCE AND THE RELATIONSHIPS OF THE EXPERIMENTAL RESULTS

Mechanism of recovery or maintenance of systemic blood pressure after spinal transection: The amount of recovery of blood pressure. If the blood pressure is measured some time after transection of the cord, it is not possible to ascertain from such measurement alone the factors which are operating to maintain the level of pressure found. Since this is so, it becomes necessary to resort to some other experimental procedure which will result in a further analysis of these factors. Any conclusion

regarding the mechanism for the recovery or the maintenance of blood pressure must be based on the experimental results obtained. Therefore it is necessary to consider the bearing of the results on this problem.

The exact amount of recovery of blood pressure is difficult to determine. The literature contains no systematic information as to the average blood pressure after transection of the cord at different levels. However, two lines of evidence present themselves as criteria on which to base the assumption that the blood pressure is higher after an interval of recovery than immediately after the transverse lesion is made. These are the unpublished experiments of C. S. Winkin (11) done in the Columbia laboratory and the condition of the animals in my series observed daily from the time of operation until the measurement of blood pressure was made.

Mrs. Winkin found a significantly lower pressure in cats in which the lesion was made in the region of the second and third thoracic segments than was observed in the present series of experiments where the blood pressure was measured some days after the transection. Furthermore, in my series, immediately after the operation, the animals gave every indication of weakness. They showed no inclination to move about but lay quietly in the pan. The body temperature was subnormal. They manifested no interest in food. Within a day or so the picture changed. They had become sufficiently active so that confinement in a cage was necessary. The body temperature rose and food was consumed with apparent relish. It is also significant that the cats with high transection showed very little recovery in the post-operative period—in fact, several died—and the level of blood pressure in those that lived was low. In every case of unusually low blood pressure before anemia of the brain, there were concomitant manifestations of indisposition.

Evidence for central mechanism. The precise extent of recovery is more or less immaterial—even the question as to whether or not any recovery has occurred is, from the point of view of these experiments, of secondary importance. That a certain level of blood pressure is being maintained after transection of the cord and that this pressure is ultimately sufficient for the performance of the usual metabolic processes is indubitable. The matter of prime importance is the central mechanism which is responsible for the maintenance of this pressure, whatever it may be. Does it lie in the medulla oblongata or is it to be found in the cells of origin in the spinal cord?

The evidence for the location of the central mechanism in the medulla lies in the results obtained by ligation of the head arteries, by stimula-

lation of certain afferent nerves before and during cerebral anemia, and in the effect of excision of the stellate ganglia or section of the extrinsic cardiac nerves before and after occlusion of the head arteries.

It is obvious that the occurrence of a significant rise of blood pressure on ligation of the head arteries is undoubted evidence that the medulla oblongata is able to affect either the rate of the heart or the caliber of the blood vessels, or both. Control experiments have shown that the mere mechanical effect of shutting off the circulation to the head, in the absence of any functional activity of the medulla, is to bring about only a trifling rise of blood pressure. Other control experiments have shown that the change in the caliber of the blood vessels is the most important agent in bringing about this rise. For it occurs in undiminished intensity when the extrinsic cardiac nerves are divided and the vasomotor nerves intact. (See cat 7 a.) Also the rise is small when the cardiac nerves or the nervi accelerantes, at least, are intact, but the thoracic ganglionic chain divided at the level of the diaphragm. (Unpublished results of Mrs. C. S. Winkin.) The occurrence of the usual anemic rise of blood pressure in cats some days after complete transection of the spinal cord is strong evidence, therefore, that the central vasomotor mechanism in the medulla oblongata can exert some influence upon the caliber of the splanchnic blood vessels. This is in direct contradiction to Goltz's statement, for which he gave no experimental proof, that the portion of the nervous system lying above the level of transection had nothing to do with the recovery or maintenance of blood pressure in the region below the level of the spinal transection.

Effect of stimulation of afferent nerves. Stimulation of several strands of the brachial plexus before functional elimination of the medulla resulted in a rise of pressure, which disappeared with the cessation of bulbar function. It may be said that the failure of the usual vasomotor response of the brachial nerves was due to a "diaschisis" effect on the cells of origin located in the cervical cord following paralysis of the bulb. But even if such a diaschisis effect is possible—though far from probable—the mere occurrence of a reflex vasomotor response on stimulation of the brachial would indicate that some mechanism lying above the level of transection was exerting some influence on systemic blood pressure.

A fall in blood pressure on stimulation of the central end of one vagus, the other being intact, may be brought about in two ways:—*a*, by the cardio-inhibitory action of the intact vagus, or *b*, as a result of stimulation of depressor fibers which accompany the vagus in the cat. The inhibi-

tory action of the intact vagus was probably the cause of the fall in the majority of cases herein cited. But the fall in pressure which was obtained by stimulation of the central end of one vagus after both had been sectioned may be explained by the action of the depressor. The effect of the depressor, however, involves an action upon the blood vessels of the splanchnic region. The evidence obtained from stimulation of the central end of the vagus points in the same direction as that obtained from stimulation of the brachial. But in the case of the vagus, its known anatomical relations indicate that the reflex pathway passes through the medulla oblongata, and the complete failure of the response after paralysis of the medulla reveals the necessity of a central connection through the medulla, and the prime importance of the bulbar mechanism in the control of cardio-vascular reactions.

Effect of excision of the stellate ganglia or section of the nervi accelerantes. It is apparent from the evidence presented that the vasomotor mechanism is a more powerful agent in bringing about changes of systemic blood pressure, under most conditions at least, than the extrinsic cardiac nervous mechanism. But the rôle of the extrinsic cardiac nerves and particularly the accelerators in compensating for changes in the caliber of the blood vessels comes out prominently under other experimental conditions. The failure to obtain a large rise of blood pressure on occlusion of the head arteries of the cats with transected spinal cords after section of the accelerators or excision of the stellate ganglia calls attention to the rôle of the accelerators. In the case of excision of the stellates, the failure of the usual rise on occlusion of the head arteries might be due to the interruption of the nervi accelerantes or to the interruption of vasomotor fibers passing through the stellate ganglia. It seems probable that the nervi accelerantes are an important part of this compensatory mechanism since merely cutting all cardiac branches from the stellates results in a failure to obtain a rise.

The resemblances or differences in deportment of animals with intact extrinsic cardiac nerves, but with transected spinal cord and impaired vasomotor system, as compared with animals with intact spinal cord and vasomotors but with division of the extrinsic cardiac nerves, can be extended still farther. Animals in which the extrinsic cardiac nerves have been divided exhibit no discomfort when at rest, but are unable to undergo any considerable exertion (12). One sometimes sees in the textbooks (13) the statement that the splanchnic vessels contract while the peripheral vessels to the somatic musculature dilate on muscular exertion. The facts brought out in this paper show that the splanch-

nic blood vessels may, by the narrowing of their lumen, bring about a great rise of blood pressure. If any significant amount of contraction of the walls of the splanchnic vessels actually occurred, one would expect a sufficiently high blood pressure to permit an animal deprived of its cardiac regulatory nerves to maintain a considerable degree of activity. The question apparently needs further study.

The effect of asphyxiation: The mechanism of the asphyxial rise. The asphyxial rise of blood pressure occurring at the close of the experiment is of considerable interest. To what can this asphyxial rise be attributed? Obviously, it must be due to an increased heart action or to vasoconstriction, in response to the accumulation of carbon dioxide in the blood. Examination of the records reveals no increase in heart action either in rate or force. Indeed, the beat becomes progressively weaker. The vasomotor mechanism, then, must be responsible for the effect. The vasoconstrictor center had been paralyzed for some time; therefore it is hardly conceivable that it could be further affected by the excess of CO_2 in the general circulation. This leaves the possibility of stimulation of cells of origin of efferent vasomotor fibers in the spinal cord or of direct stimulation of the vascular musculature. Apocodeine administered intravenously (14) abolishes the asphyxial rise. According to Dixon, apocodeine paralyzes central cells in the spinal cord. In view of these facts it appears that the asphyxial rise of blood pressure on cessation of artificial respiration is due to the stimulation of the spinal cells of origin of vasomotor fibers by carbon dioxide. Here again we find cells in the isolated portion of the spinal cord responding to a stimulus and this a "natural" stimulus. Could they react in this way if they were really inhibited by shock? On the contrary this response furnishes additional evidence of the influence of the bulbar centers since the cells of the spinal cord are still fully able to function. The results so far obtained indicate that the activity of these spinal cells is dependent on nerve impulses from higher centers. Nor apparently do they discharge efferent impulses over vasomotor fibers in response to afferent impulses from outside the central nervous system. Usual spinal reflex arcs are open and functional, e.g., stimulation of the sciatic produced a marked muscular contraction of the hind leg, sometimes of both hind legs. If, then, afferent pathways from the viscera to the spinal vasomotor cells existed, it would not be unreasonable to suppose that vasomotor changes could be brought about and blood pressure maintained through reflex action involving only the lower centers.

The duration of the heart beat. The duration of the heart beat in asphyxia in all probability bears a direct relationship to the rapidity of accumulation of carbon dioxide in the blood, and this, in turn, is related to the degree of activity of the skeletal muscles. It is known that the duration of the heart beat in asphyxia after paralysis of the skeletal muscles by curare is much greater than when no curare has been given, extending at times over a period of an hour. The significantly longer duration of the heart beat in asphyxia in the cats of this series as compared with normal cats indicates a decreased activity of the skeletal muscles. The relation of length of the period of recovery after the first operation to the duration of the heart beat in asphyxia is not clear from this series of experiments.

Rigor mortis. The observations on the duration of the heart beat in asphyxia are in line with the delayed onset of rigor mortis in the hind limbs after spinal transection. Both series of facts indicate a decreased activity of the skeletal muscles under these conditions.

Incidental observations: Ineffectual labor. Goltz (15) cites two instances in which labor ran its normal course after transection of the spinal cord. In the first case conception, pregnancy and parturition occurred after the cord had been cut at the level of the first lumbar vertebra. Nearly 8 months later the dog gave birth to one live puppy. Two dead fetuses were removed and the bitch died 2 days later of peritonitis. In the second case the cord was transected at the level of the third thoracic vertebra and 3 days later 94 mm. of the lumbar cord were removed. Four hours after this second operation, the first of five live puppies was delivered. The duration of labor was about 30 hours. The bitch lived nearly 2 months after the birth of the pups. The cause of the failure of a normal delivery in the case of the cat, 10 days after spinal transection at the level of the third thoracic vertebra, is not clear.

Certain aspects of the relation of the nervous mechanism to the regulation of physico-chemical conditions in the animal organism upon which these results have a bearing may be indicated here. Because of lack of time the observations were not made during the course of the experiments, but the problems may be attacked more specifically at some future time. It is commonly stated that the secretion of urine ceases when the systemic blood pressure falls below 30 or 40 mm. Hg. This raises the question whether the death of some of the animals with transection of the spinal cord at the level of the seventh cervical segment may not have been due to uremia, due to lack of secretion of urine. In a few instances, the bladder was found practically empty at autopsy, but

usually there was sufficient urine to allow of its expression during the life of the animal. No analyses of blood were made in this connection, and therefore no definite answer to the question can be given now. The known relation of low systemic blood pressure to a low concentration of carbon dioxide in the blood raises the further question as to the concentration of carbon dioxide in the blood of the animals used in this research during the period of recovery. No specific observations were made, and the answer to the question must be deferred until further experiments are done. Further observations of this nature are needed to bring out the relationship of the nervous system to the physico-chemical regulation of the organism. If, as there is every reason to suppose, the law of mass action applies to many of the reactions in the living organism, any lesion of the nervous system which modifies in any way the conditions of pressure in the organism might be expected to have its effect on some metabolic process (16). The results now in the literature are sufficient to indicate that this possibility is, in some instances at least, a reality.

CONCLUSIONS

1. Recovery of systemic blood pressure occurs after an interval in cats in which there has been complete transection of the spinal cord, if the level of transection does not lie above the level of outflow of the thoracic sympathetic chain.

2. The extent of the recovery of blood pressure depends largely upon the level at which the transection is made and partly upon the length of time allowed for recovery. In a few cases the recovery was nearly complete.

3. The mechanism through which the recovery is brought about includes the cardio-vascular mechanism in the medulla, and the outflow from the center which includes the extrinsic cardiac nerves and the vasomotor nerves. The vasomotor portion of this outflow is of the greater importance.

4. Spinal cells of origin of efferent vasomotor fibers lying below the level of transection do not take on, within the time limits of these experiments—2 days to 32 days—any significant part of the burden of maintaining the systemic blood pressure.

5. The experimental results lend no support to the view that there are spinal vasomotor centers functionally active and coördinate in rank with the mechanism in the medulla oblongata. They do support the

view that the bulbar vasomotor mechanism is the chief mechanism upon which the functional activity of all other parts of the vasomotor system depends.

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THE EFFECT OF HEMORRHAGE ON THE SYMPATHETIC NERVES

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We have observed, as others (1) have, that hemorrhage not only produces hyperglycemia, but also that it sensitizes or stimulates the sympathetics as judged by the response to injections of adrenalin or nicotine. This is shown by the greater blood pressure rise, caused by the injection of a constant amount of adrenalin or nicotine before and after hemorrhage. The present investigation is an attempt to analyze the effect of hemorrhage on the nervous system, and to determine whether the action is central or peripheral, or on both locations; and secondly, to determine whether the increase in blood sugar is the only result of sympathetic stimulation or whether the hyperglycemia in turn influences the sympathetics.[1]

The physical changes in the vasomotor response have been investigated especially by Porter (2) and by Sollmann (3), but they do not agree on interpretation. Porter thinks the percentile rise in blood pressure is the true measure of the vasomotor response, while Sollmann and Pilcher say that the absolute rise is the more correct index. Neither method is wholly satisfactory and neither investigator analyzes the cause of the rise in blood pressure, but assumes it to be an action on the center. In this and similar work, the vasomotor response cannot be correctly interpreted as an action on the vasomotor center, but merely on the vasomotor mechanism, and the peripheral part of this mechanism may be more important than the central. Adrenalin, for example, raises the blood pressure but it has practically no influence on the vasomotor center. In the case of hemorrhage in particular we think most of the influence is peripheral. The present work supports this opinion. However, it is highly improbable that any action on nerves is confined absolutely to one location, though it may be mainly local. For example,

[1] The relation of increased sugar and epinephrin and nicotine action is being investigated by Drs. P. G. Albrecht and J. T. Groot.

while the action of curara is mainly peripheral it has also a central action (4), and while strychnine acts mainly on the cord, it may also have a peripheral action; so also the effect of hemorrhage on the sympathetics can hardly be expected to be confined absolutely to the periphery although its greatest action is there. Here again much work on the vasomotor center is needlessly complicated by the use of curara, and while Sollmann (3) justifies its use in some cases he found that it acted on the vasomotor center. McGuigan (4) has also shown that curara has a central action, and Reid Hunt (5) found after large doses of curara a reflex pressure rise may be absent.

That the effect of hemorrhage on the sympathetics is mainly peripheral, however, we think is supported by the following facts and experiments:

During anesthesia the rise in blood pressure after adrenalin is less than if no anesthetic had been given, and anesthetics depress the centers mainly (cf. with strychnine which stimulates these centers).

This might be taken to prove that the greater action of adrenalin after hemorrhage is due to central action. However such an assumption ignores the important fact that anesthetics depress also the peripheral mechansim. It is obligatory, therefore, in the analysis of this problem to stimulate or depress the centers independently of the peripheral mechanism. This we have done.

We have a method of stimulating the central parts of the nervous system without directly involving the peripheral parts. It is well known that many drugs injected into the cerebrospinal fluid are but slowly absorbed and therefore, for a long time, act locally. Taking advantage of this fact, we can stimulate the central system with strychnine, caffeine, cocaine, etc., or depress it by chloral, chloroform, etc., independently of the peripheral parts. In such cases it is found that when the centers are highly sensitized, the effect of adrenalin is no greater than before and that depression of the centers also has little influence.

The following experiment is illustrative:

Dog 8 kilos—1 cc. adrenalin 1:10,000 intravenously.

Normal blood pressure	*Blood pressure after adrenalin*
160 mm. Hg.	225 mm. Hg. rise—65 mm. Hg.
160 mm. Hg.	230 mm. Hg. rise—70 mm. Hg.

Strychnine was now injected into the 4th ventricle until a definite twitching of the head was elicited. The blood pressure was now—

Normal pressure 220, after adrenalin 260, rise 40 mm. Hg.
Normal pressure 220, after adrenalin 260, rise 40 mm. Hg.
Normal pressure 220, after adrenalin 260, rise 40 mm. Hg.

Similar results are obtained whether the vagi are cut or intact.

Here it is seen that stimulation of the anterior part of the central nervous system causes no increase in the effect of adrenalin or even lessens the effect. The charge may be made legitimately, that strychnine in this region does not stimulate part of the cord from which the major part of the sympathetic arises. This may be true, and to make the test complete, we have strychninized the region below the medulla with the following result:

A dog 10 kilos—under ether had a blood pressure of 110 mm. Hg. After 50 cc. hemorrhage this rapidly dropped to 40 mm. Hg.; 1 cc. 1:10,000 adrenalin now caused a rise of blood pressure to 140 mm. Hg. or 100 mm. rise. The blood was defibrinated and most of it was injected into the femoral vein. This raised the blood pressure to 80 mm. Hg. One cubic centimeter adrenalin (1:10,000) now raised the pressure to 165 mm. or a rise of 85 mm. Hg. This however soon fell to 40 mm. again. At this point the sympathetic region of the animal (dorsal) was strychninized until definite twitching in this region occurred. One cubic centimeter adrenalin now caused a rise of blood pressure to 70 mm. Hg. Apparently, therefore, central strychninization does not increase vasomotor response to adrenalin.

Another experiment with the whole animal under the influence of strychnine, shows that central stimulation with adrenalin does not increase the pressure.

Dog 15 kilos—ether anesthesia. Normal blood pressure 100 mm. Hg. The same volume of adrenalin was used in all cases. One cubic centimeter 1:10,000 adrenalin intravenously raised blood pressure to 145 mm. or a rise of 45 mm. Hg.

Strychnine was now given until definite twitching occurred after which three separate injections of 1 cc. adrenalin raised the pressure to 145 mm. Hg. or an average rise of 45 mm. Hg. There was no spasm from the strychnine, but merely twitching. The animal was now bled 100 cc., this lowered pressure to 90 mm.

Adrenalin now raised it to 130 mm.
Adrenalin now raised it to 140 mm. or
An average rise of 45 mm.

Another hemorrhage of 150 cc. lowered the pressure to 55 mm. Hg.

Adrenalin now raised pressure to 105 or 55 mm. rise. The animal was again bled 80 cc. This lowered the pressure to 35 mm. Adrenalin now raised the pressure to 78 or 43 mm. Hg. rise. In this experiment adrenalin caused the same rise after strychnine as before. In other cases we have found a lesser rise after strychninization. Hemorrhage in this case again caused a sensitization to adrenalin action. Since the centers were apparently stimulated to the maximal by strychnine the action of hemorrhage seems to be peripheral.

As is well known, stimulation of the central end of a peripheral nerve raises the blood pressure. This is taken as an action on the vasomotor

center. The degree of rise depends on the condition of the center. If, therefore, we stimulate or depress the center, we vary the response to sciatic stimulation accordingly. If, then, the condition of the central nervous system changes the vasomotor response to those influences which we know act on the center and does not change the response to adrenalin, we are justified in assuming that adrenalin does not act on the center. In addition, if adrenalin is more effective after hemorrhage we are justified also in assuming that hemorrhage acts peripherally on the nervous system. However, when the region of the fourth ventricle is fully strychninized stimulation of the sciatic causes no greater rise of blood pressure than before, unless some tetanus occurs. This means that the vasomotor mechanism is but little acted on by strychnine and the method does not enable us to settle the question.

The effect of cocaine on the cord. It is well known that the action of cocaine and epinephrin are synergistic. Such synergism is manifestly proven only for the periphery. Since cocaine acts centrally also, it may be assumed that if adrenalin acts centrally there is also a central synergism; but if sufficient cocaine be given to eliminate conduction in the cord, this would manifestly eliminate all possible synergistic actions.

To positively place cocaine in the cord in several cases we laid it bare by operation. In addition to permitting the placing of cocaine on the cord the operation in this case had the desirable effect of lowering the blood pressure. This can be considered as a hemorrhage into the blood vessles. We have therefore the effect of hemorrhage and the removal of central influence. Under such conditions the central action of cocaine does not change the response to adrenalin. We can, therefore, assume that hemorrhage in this case acts more peripherally than centrally. The following experiment shows the effect of hemorrhage and cocaine. All hemorrhages, however do not give an absolute blood pressure increase to adrenalin but all do give a percentile increase, many give the same percentile increase and some a greater absolute increase.

Dog 10 kilos. Tracheal cannula, vagi cut. Adrenalin, 1 cc. at each injection 1:20,000. Pressure expressed in mm. Hg.

Normal pressure 146. Adrenalin raises it to 210, 206, 210, average rise = 63.	
After 60 cc. hemorrhage—the pressure is	135
Adrenalin now raises pressure to	202, rise = 67
After another hemorrhage of 45 cc. pressure	114
Adrenalin raises to	196 or 82 rise
5 cc. 0.5 per cent cocaine in 4th ventricle stops respiration and reduces pressure to	82
1 cc. adrenalin now raised pressure to	156 or 74 rise
Cocaine 5 cc. intravenously pressure now	116
Adrenalin raises pressure to	226 or 110 rise

The hemorrhage here lowers the blood pressure, but the adrenalin is more effective, and this means stimulation of the vasomotor mechanism. Stimulating the center with cocaine does not increase effectiveness of adrenalin, but the peripheral action of cocaine enormously increases the effectiveness. We think, therefore, since the condition of the center seems not to change the vasomotor response, that the effect of hemorrhage is peripheral.

I. Action of nicotine

Young dog—weight 5 kilos—vagi cut—tracheal cannula for ether. 1 cc. 1:2,000 adrenalin used in each case. Normal blood pressure 70.

Rise with adrenalin—58—in three cases. Nicotine was now given intravenously until no blood pressure rise was obtained on last injection.

The blood pressure is..... 78
Adrenalin now raises pressure in 2 administrations, 94 and 100 or an average rise of..... 97

which is almost twice the normal rise before nicotine.

This would indicate that the central effect of adrenalin, if any, works against a rise of pressure, and favors the view that the action of adrenalin after hemorrhage is peripheral.

II. Action of nicotine

Dog—weight 15 kilos. This animal had 6 grams of chloral hydrate 4 hours previously. Adrenalin used 1:20,000. Tracheal cannula inserted for anesthesia, and vagi are cut.

Normal blood pressure..... 176
Rise by 1 cc. adrenalin..... 48

After paralysis of the vasomotors by nicotine, 1 cc. adrenalin caused a rise of 84 or almost twice the normal rise.

III. Action of nicotine

Dog—weight 14 kilos. Nicotine 1–4000, was used intravenously, since paralysis of the ganglions was not desired. Carotid pressure—vagi cut—ether anesthesia.

Normal blood pressure..... 204
1 cc. nicotine raises pressure to..... 210 or a rise of 6
60 cc. blood withdrawn, reduces pressure to..... 182
1 cc. nicotine now raised it to..... 198 or a rise of 16
93 cc. blood withdrawn, lowers pressure to..... 156
Nicotine now raises it to..... 198 or a rise of 42

IV. Action of chloral centrally

Dog—weight 12 kilos. Nitrous oxide anesthesia—no ether used. Vagi cut. Adrenalin used 1:40,000, 1 cc. at each injection. Normal pressure 192.

Adrenalin raises to 252 or a rise of 60.

Bled 100 cc. which lowered pressure to 156.

Adrenalin raises pressure to 218 or a rise of 62.
Adrenalin raises pressure to 222 or a rise of 66.
4 cc. chloral hydrate (2 per cent) into 4th ventricle after which the pressure is 168.
Adrenalin raises pressure to 230 or a rise of 62.
Animal bled again—100 cc. which lowered pressure to 100.
Adrenalin raises to 158 or a rise of 58.

Summary. The effect of chloral into the 4th ventricle sufficient to anesthetize the animal deeply, is without apparent effect on the reaction to adrenalin.

V. Action of chloral

May 12, 1921. Dog weight—10 kilos—no ether or vol. anesthetic. Time: 1:42; 5 cc. 10 per cent chloral hydrate into 4th ventricle gives immediate anesthesia. This was the only anesthetic used.
At 1:50: Tracheal carotid and femoral cannulas inserted and vagi cut.
Adrenalin used 1 cc. 1:80,000 at each injection.

Normal rise caused by adrenalin	93
Normal presure 95, but it remains at 126 after three injections of adrenalin.	
Bled 58 cc. reduces pressure to	118
Adrenalin now raises pressure to	222
or a rise of	104
Bled 80 cc. reduces pressure to	108
Adrenalin now raises the pressure to	216
or a rise of	108
Further bleeding of 100 cc. reduces the pressure to	62
Adrenalin now raises pressure	128
or a rise of	66

In this case, the nerve endings are not anesthetized and after hemorrhage adrenalin causes a greater rise, until the hemorrhage is so great that the vessels are too flaccid to give an increase rise. Even then the percentile rise is greater than the normal. Such cases as this render it unnecessary to discuss whether the percentile or absolute rise is the proper method of interpretation, since both are increased.

DISCUSSION

The present investigation was suggested from work on the influence of the nervous system on the blood sugar. In that work it was found that all nervous impulses that increase the sugar in the blood pass over the sympathetic nervous system. Others have also reached this conclusion. The proof of this is briefly: stimulation of the motor areas does not increase blood sugar. Piqûre does not increase it if the splanchnic nerves have been severed; stimulation of sensory nerves may or may not give a hyperglycemia; similarly, neuritis of various forms is only

rarely accompanied by hyperglycemia. Nicotine does not increase blood sugar after adrenalin; in other words, stimulation of the parasympathetic ganglions only has no effect; similarly pilocarpine and atropine are without significant influence on the blood sugar (6). Therefore, the sympathetics are the important nerves in blood sugar regulation.

Since hemorrhage also causes hyperglycemia it seems that it stimulates the sympathetics, and since strychnine may be used to free the liver of glycogen its use in the present problem was suggested as probably also acting on the sympathetics.

The objection may be made that strychnine sensitization of the cord does not involve the sympathetics. However in addition to the above citations there is a considerable amount of evidence that it does. Hefftter (7) cites proof of an action on the vegetative system and it is well known that strychnine will free the glycogen from the liver, and since all impulses increasing the blood sugar pass over the sympathetics strychnine may be correctly used to sensitize the central nervous system in the present problem. The method is especially justified since strychnine in the doses used strongly stimulates the vasomotor center (4), and has very little peripheral effect.

Hemorrhage might conceivably increase the secretion of adrenalin into the blood and at least partly account for the increased irritability. However, Hoskins and Rowley (8) found that no concentration of adrenalin injected into the blood augments vasomotor irritability, and sometimes lessens it. This depression they think is probably both central and peripheral. We have noticed, however, that the first, sometimes also the second and third injection of 1 cc. 1: 20,000 adrenalin solution is less effective in raising the blood pressure in anesthetized animals than in succeeding injections, which may mean some sensitization. With Rosser, Hoskins and Rowley (1) studied the effect of hemorrhage on vasomotor irritability, and found it increased as judged by the response to nicotine. The increase they think is confined to the vasomotor center. It is questionable, however, whether the method used is adequate. They used adrenalin, the concentration (1:100,000) which is perhaps too weak to indicate small changes; with this concentration they found no increased irritability in the animals which were either under ether anesthesia or decerebrate. In the latter group the low concentration of adrenalin used would be much more effective. It is well known that in chemical titrations some indicators are not sufficiently delicate to show considerable changes in the concentration of hydrogen ions, so adrenalin in this dilution may be inadequate to indicate a change in this case.

With nicotine they obtained positive results which we can confirm, and since the results (peripheral) with adrenalin were negative, they conclude the positive effect of nicotine was probably due to increased central irritability. However, while nicotine (4) stimulates the vasomotor center the main action is on peripheral ganglion cells and the effect recorded in these experiments was apparently on the sympathetic ganglia and, therefore, peripheral and in agreement with our own.

By measuring the rise of blood pressure on stimulation of the central end of the sciatic or brachial nerve, Porter (9) has shown that the tone of the vasomotor center is little affected by hemorrhage. In arriving at this conclusion he lays stress on the percentile rather than the absolute rise, as the true index of the condition of the vasomotor cells. When the percentile rise is taken as the index there is a stimulation of the vasomotor center. This fact if accepted has little bearing on the present problem except in confirming the opinion of Hoskins (8). Sollmann states that the absolute rise is a more reliable criterion of the response of the vasomotor center than is the percentile rise, and recently Crile (10) has pointed out the weakness of Porter's logic. Stimulation of a peripheral nerve, however, as admitted by all who have used it, is an unsatisfactory method of determining the condition of the center. In the present investigation we have to a great degree eliminated the necessity of discussion over the relative values of percentile and absolute changes, since in many cases both are changed.

If we look at blood pressure as we do at balanced reaction which tends to reach an equilibrium at a definite height in the reaction, we should expect a greater percentile rise when the pressure is low. If this is not permissible, it certainly is obvious that the same force exerted at a low pressure should be more effective in raising the pressure than if it were exerted at a higher level. Now *ceteris paribus*, stimulation of the vasomotor center should increase the effectiveness of adrenalin on the blood pressure, because it is known that adrenalin is more effective without anesthesia, which depresses the centers; but great weight cannot be given to this because a general anesthetic also depresses the vasomotor endings which is the main point of action of adrenalin. While adrenalin may act on the center directly such an action can be shown only with especial technique (11), (12), (13), (14) and from the results cited we think the central stimulation after hemorrhage is of less importance than the peripheral effect. The peripheral action of adrenalin seems less in many cases after strychnine and other central stimulating drugs. This may be similar to the action of the brain on the lower motor neu-

rons, i.e., the influence of the center restrains peripheral action. Similarly after nicotine, which removes all central influence, adrenalin is a more powerful vasoconstrictor.

SUMMARY

Hemorrhage in many cases sensitizes or stimulates the sympathetic system governing vascular tone. By the use of drugs that are known to stimulate or depress the centers and which have a lesser or no effect on the periphery, it is shown that the greater influence of the hemorrhage on the vasomotor mechanism is peripheral.

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THE CHANGES IN THE CONCENTRATION OF THE CARBON DIOXIDE RESULTING FROM CHANGES IN THE VOLUME OF BLOOD FLOWING THROUGH THE MEDULLA OBLONGATA

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Attention has been called to the changes in respiration in dogs following the permanent ligation of the two carotid and two vertebral arteries as in Sir Astley Cooper's experiments (1), (2). A question asked by a friend as to the effect of changes in respiration consequent upon changes in the volume of blood flowing through the medulla oblongata led to the repetition of these experiments in a modified form, with cats as the experimental animals, and temporary instead of permanent ligation of the arteries, and the estimation of the carbon dioxide in the arterial blood under the various conditions of blood flow through the medulla. A preliminary note has been published, giving some of these changes in the gases of the blood (3).

The experiments reported here were made on cats. Ether and tracheotomy were routine procedures. Blood pressure was taken by a mercury manometer connected with one carotid artery. The other carotid was cleaned from its surrounding tissues and loosely ligated. The two vertebral arteries were isolated before their entrance into the foramina of the transverse processes of the cervical vertebrae and loosely ligated. Arterial blood for analysis was drawn from one femoral artery and centrifuged without exposure to the air. The carbon dioxide content of the plasma was determined by the Van Slyke method. The respiratory movements were recorded by a Crile stethograph connected with a Verdin tambour. Samples of blood were taken for analysis before placing clamps on the arteries, while the clamps were on, and after their removal. Usually 10 cc. of blood were drawn at one time.

The effect of temporarily clamping the four arteries is an increase in magnitude or rate of the respiratory movements, and a marked rise of the arterial blood pressure. During the period of increased respira-

tory activity, the concentration of carbon dioxide falls in the arterial blood. (Tables 1 and 2.) When the flow was long obstructed there was, of course, an accumulation of carbon dioxide in the blood as the second sample in table 2 indicates. When the circulation to the head is restored, the blood pressure promptly returns to normal, and the

TABLE 1

DATE	NUMBER OF OCCLUSIONS	AVERAGE CO_2 CONTENT OF PLASMA IN VOLUMES PER CENT	
		Before occlusion	After occlusion
1- 6-19	4	31.1	30.4
1- 8-19	4	46.9	41.4
1-10-19	4	46.8	43.0
12-18-18	2	55.0	45.0
12-21-18	2	41.0	38.0
12-26-18	4	49.8	44.7

Only samples obtained before 40 per cent of the blood had been drawn are included in this table.

TABLE 2

Experiment 1-10-19

NUMBER OF SAMPLE	CO_2 CONTENT OF PLASMA IN	EXPERIMENTAL PROCEDURE	NUMBER OF SAMPLE	CO_2 CONTENT OF PLASMA IN	EXPERIMENTAL PROCEDURE
	volumes per cent			*volumes per cent*	
1	49.0	Anesthesia	9	46.0	Obstruction continued
2	52.5	Flow long obstructed	10	44.7	Free flow; 44 per cent blood drawn
3	48.5	Free flow	11	38.0	Flow obstructed
4	46.0	Obstruction to flow	12	35.0	Free flow
5	48.0	Free flow	13	31.1	Flow obstructed
6	43.5	Obstruction to flow	14	31.5	Obstruction continued
7	46.0	Free flow	15	24.7	Flow obstructed
8	44.5	Obstruction to flow	16	24.7	Free flow

respiratory movements resume their usual rate and amplitude, or closely approach normal.

Occasionally clamping all four arteries results in too great restriction of blood flow to the medulla oblongata, and the first great increase in respiratory movements is soon followed by a slowing or stoppage. In such cases, one vertebral artery may be released. As successive samples

of blood are withdrawn for analysis, resulting in a diminution of the volume of the circulating fluid, closure of one carotid artery, leaving both vertebrals open, may be sufficient to produce the desired change in respiration.

The changes in blood pressure and respiratory movements are shown in figure 1, and the changes in the concentration of the carbon dioxide in the blood plasma are given in table 2.

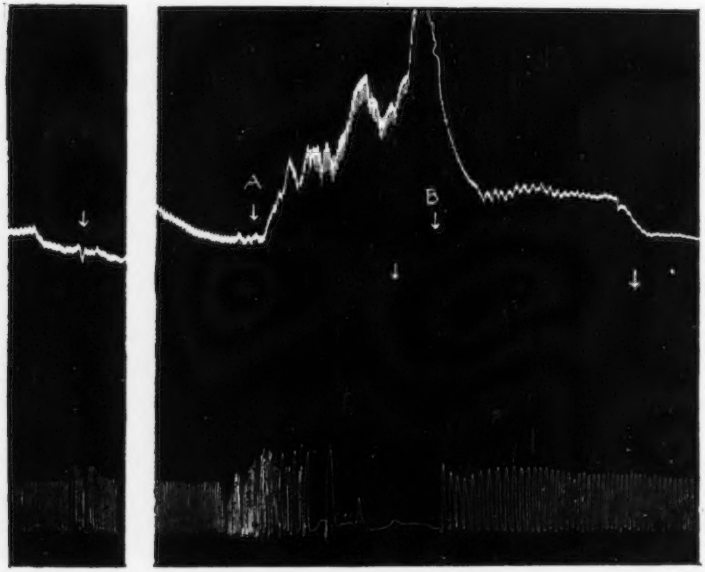

Fig. 1. Upper tracing shows blood pressure taken from one carotid artery. The lower tracing shows costal respiratory movements. The figures below show the concentration of carbon dioxide in the plasma in 10 cc. samples of the blood drawn from the femoral artery at the time indicated by the arrows. The small section at the left of the figure is a control. The concentration of carbon dioxide in the blood was 44.7. Both vertebral arteries had been ligated. At *A* the remaining carotid artery was clamped. The blood pressure rose sharply and the respiratory movements increased. The clamp was removed from the artery at *B*. The blood pressure fell rapidly but remained above normal for a time. A further fall occurred on drawing the last sample of blood. Nine previous samples of blood had been drawn, and the combined effect of hemorrhage and restriction of the circulation to the medulla led to a slowing of respiration after the first marked increase. Respiratory movements were resumed as soon as the circulation to the medulla was restored.

The central respiratory mechanism is sensitive to changes in the volume of blood, since changes in the concentration of the carbon dioxide may appear before the volume of blood withdrawn has begun to affect the systemic blood pressure in any marked degree.

The explanation of the results apparently depends upon two conceptions. First of all, it appears that conditions in the central mechanism itself determine respiratory activity. For it does not appear possible to explain the variations in rate and amplitude of the respiratory movements simply on the basis of the concentration of carbon dioxide in the arterial blood plasma. If the respiratory activity were dependent upon mere concentration of the carbon dioxide in the blood, then one would expect a decrease in rate or amplitude of the movements when the concentration of the carbon dioxide falls. But this is not in accordance with the facts, for the decreased concentration of carbon dioxide in the blood is the result of increased pulmonary ventilation. This increased pulmonary ventilation must result from the change in conditions in the cells of the medulla oblongata itself.

The second conclusion relates to the application of the law of mass action to conditions in the central cells of the nervous mechanism for respiration. If the activity of the central cells from which the efferent impulses in respiratory movements arise depends upon the concentration of hydrogen ions or of carbon dioxide in the cells themselves, we have next to inquire how the volume of blood flowing through or past these cells is related to the conditions in the cells themselves. If the volume of blood flowing through the medulla oblongata in unit time is reduced, each cubic centimeter of blood must take up more carbon dioxide than when the volume of blood flowing through the center is normal. But an increase in the amount of carbon dioxide in each cubic centimeter of blood coming from the central mechanism would inevitably mean a rise in the concentration of carbon dioxide in the nerve cells themselves as a direct result of the solubility coefficient. The direct result of the increase in the concentration of carbon dioxide in the nerve cells would be an increased rate or amplitude of respiratory movements. This would lead to a reduction in the carbon dioxide in the blood. The process would tend to continue until the carbon dioxide in the blood coming to the cells of the medulla was reduced to such an extent that the additional carbon dioxide taken up by each cubic centimeter of the blood leaving the respiratory center did not bring the total concentration of carbon dioxide per cubic centimeter above normal. It is only by such an adjustment that the concentration of carbon dioxide in the cells of the respiratory center could be reduced to and kept at the normal.

The prompt return of the concentration of carbon dioxide to normal when the full volume of blood flow to the medulla oblongata is reëstablished argues against any change in the capacity of the plasma to take up carbon dioxide, as has been suggested in cases of cardiac dyspnoea, nor does there seem any probability of the occurrence of sufficient amounts of acid to account for the decrease in the carbon dioxide of the blood. The hypothesis based on the application of the mass law seems a more much probable explanation of the facts observed. The occurrence of a low concentration of carbon dioxide in cardiac dyspnoea would seem to be a case of much the same sort, as one would expect a decreased volume of blood flowing through the medulla oblongata under conditions of such grave involvement of the heart. The change in the deportment of the cells of the respiratory center is to be expected as a result of the change in the volume of blood flowing through the medulla if, as has been suggested in an earlier paper (4), the constancy of various internal conditions of the organism is to be explained on the general basis of the law of mass action as a means of regulating the degree of activity of the different functional systems of the organism. The application of le Chatelier's earlier theorem, particularly in the form in which it was stated by Riedel (5) that "when any system is in a state of physical or chemical equilibrium, a change in one of the factors of equilibrium will cause a reverse change within the system" seems clear. The volume of blood flow, as related to concentration, is one of the factors of equilibrium. The change in the reverse direction occurring within the system would be an increase in the number or intensity of efferent respiratory impulses, tending to restore the factor of concentration of carbon dioxide to its former normal value.

Changes or reactions of this type occurring within the animal organism have often been spoken of as adaptations. Treviranus, it will be recalled, regarded adaptation as one of the most important characteristics of living matter. Despite the tendency in some quarters to decry adaptation at the present day, we may possibly retain the idea in physiology for some time to come. As has been indicated, we are inclined to regard it as a case under le Chatelier's theorem. And since adaptive reactions are common in higher animal forms, we may find some of the best and most striking illustrations of a general principle of physical chemistry in these forms. Furthermore, we may, by the aid of this general principle, arrive at some of the generalizations of which physiology at the present day stands so greatly in need.

Similar considerations apply to the rise of blood pressure during the period of restriction of the circulation through the medulla. A general

rise of blood pressure would tend to force more blood through the remaining blood vessels than would flow through them under ordinary systemic blood pressure. This would, in its turn, tend to remove more completely any excess of carbon dioxide accumulated in the cells or fluids of the medulla oblongata and, what may be quite as important, convey more oxygen to them. The restriction of the volume of blood flowing through the medulla leads to a change in the system in the reverse direction. This change leads to an increase in the volume of blood flowing through the system.

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THE ACTION OF NEUTRAL ISOTONIC SALT SOLUTIONS IN SENSITIZING ARBACIA EGGS TO THE ACTIVATING INFLUENCE OF HYPERTONIC SEA-WATER

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Some years ago the senior author found that brief exposure (5 to 10 minutes) to pure isotonic solutions of neutral sodium and potassium salts, especially thiocyanate and iodide, induced typical membrane-formation in unfertilized Arbacia eggs;[1] further, that if such eggs, soon after their return to sea-water, received the usual brief after-treatment with hypertonic sea-water, in accordance with Loeb's method of artificial parthenogenesis, a large proportion (frequently 50 per cent or more) developed to free-swimming larvae.[2] Iodides and thiocyanates were by far the most effective of the salts employed; nitrate had relatively little action, while chloride, bromide, chlorate and acetate left the great majority of eggs outwardly unaltered. The addition of a little calcium (1 mol $CaCl_2$ to 20 alkali salt) to the pure salt solution entirely prevented its membrane-forming action,[2] a similar though less pronounced effect was produced by the addition of certain anesthetic compounds (alcohols, urethanes).[3] Since both the calcium salt and the anesthetic are known to antagonize the toxic and permeability-increasing effects of pure alkali salt solutions on these eggs[4]—apparently because of their having the opposite kind of influence on the physical state of the protoplasmic surface-film—a connection of the latter effect with the membrane-forming action is indicated. A temporary increase in the permeability of the egg-surface to water and water-soluble substances appears in fact to be a regular feature of the activation-process, whether normal or artificial.[5] Apparently the formation of a visible fertilization-mem-

[1] R. S. Lillie: This Journal, 1910, xxvi, 106.
[2] R. S. Lillie: This Journal, 1910–11, xxvii, 289; Journ. Morph., 1911, xxii, 695.
[3] R. S. Lillie: Journ. Exper. Zoöl., 1914, xvi, 591.
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[5] *Cf.* R. S. Lillie: This Journal, 1916, xl, 249; 1917–18, xlv, 406.

brane is a sign that this surface change has proceeded to a certain critical stage. With membrane-formation is associated a definite change in the physiological properties of the egg; this is shown especially in a greatly increased responsiveness to the activating influence of hypertonic sea-water.[6]

The separation of a visible membrane from the egg-surface is, however, to be regarded merely as the index of some underlying process affecting the properties of the egg-protoplasm and not as the essential process itself. It was found that even when no visible effect was produced upon the eggs, their responsiveness to hypertonic sea-water was distinctly increased. This was observed in eggs that had been treated with calcium-containing solutions of iodide and thiocyanate, and also with solutions of these salts containing an anesthetic (alcohol) in concentration sufficient to suppress membrane formation.[7] It appears, therefore, that an increased susceptibility to the hypertonic treatment may result without any visible change in the eggs. Unfertilized Arbacia eggs which have been exposed to these solutions differ from eggs with definite fertilization membranes in showing no change of form or other external signs of activation when returned to sea-water, and no increased tendency to cytolysis; if left in sea-water without further treatment they exhibit no signs of alteration, but continue to live and even after 24 hours or longer respond to sperm-fertilization in an apparently normal manner.

Treatment of unfertilized Arbacia eggs with pure isotonic solutions of sodium chloride and other neutral sodium salts (nitrate, sulphate, citrate) and also—though to a less degree—with calcium-containing solutions of these salts, has been found during the past summer at Woods Hole to produce a similar increase of susceptibility to hypertonic sea-water without external signs of activation. Brief exposure to pure isotonic solutions of NaCl (0.53 to 0.54m) does not induce membrane-formation, although certain characteristic effects, agglutination and the exit of a little pigment, indicate that this solution produces a definite change in the properties of the cell-surface. Both of these effects, however, are absent in NaCl solutions containing $CaCl_2$ (e.g., 95 vols. 0.54m NaCl plus 5 vols. 0.5m $CaCl_2$); eggs treated with this and similar solutions and returned to sea-water appear quite unchanged; and even after 24 hours or longer still exhibit a normal appearance and respond

[6] *Cf.* J. Loeb: Artificial parthenogenesis and fertilization, Chicago, 1913, chapters 8 to 11.

[7] R. S. Lillie: Journ. Morph., 1911, xxii, 705 *seq.* Journ. Exper. Zoöl., 1914, xvi, 596–600.

normally to fertilization. Yet the responsiveness to hypertonic sea-water is profoundly modified by the brief exposure to the salt solution; this is true of both the pure and the calcium-containing solutions, although the effect is somewhat less in the latter case, especially when the Na-Ca ratio is 10 to 1 or greater. Even after remaining for 24 hours in sea-water after the treatment with salt solution, brief exposure (20 to 40 minutes) to hypertonic sea-water (250 cc. sea-water plus 50 cc. 2.5m NaCl) induces cleavage and development in a large proportion of eggs, many of which—usually a minority but in some cases 50 per cent or more of all eggs—develop to a blastula state. As is well known, exposure of normal untreated eggs to hypertonic sea-water for such brief periods has little or no activating effect.

Brief exposure to isotonic salt solutions thus produces a definite alteration in the physiological properties of the egg, independently of any visible surface-change like membrane-formation. This effect may be described as a "sensitization" to hypertonic sea-water. In certain respects it may be compared with the sensitization to chemical stimulation induced in irritable tissues, such as frog's muscle, by brief exposure to isotonic solutions of NaCl and other sodium salts. The essential features of this phenomenon are as follows. When a fresh normal curarized frog's muscle (gastrocnemius) is brought from Ringer's solution into a pure isotonic solution of a neutral sodium salt (NaCl, NaBr, NaI, $NaNO_3$, etc.) and left there for 3 or 4 minutes, it is found to give a much more vigorous contraction when dipped into a stimulating salt solution (e.g., 4 vols. m/8 NaCl plus 1 vol. m/8 KCl) than when it is brought into this solution directly from Ringer's solution. The muscle also shows a more pronounced response to heat, cytolytic agents, hypertonic Ringer's solution, or other agents that normally cause contractions, than it does in the normal unsensitized state. The general responsiveness to chemical stimulation is abnormally increased by the treatment with the pure salt solution; i.e., a sensitization, which is general and not specific, is induced.[8] In an analogous manner the pure isotonic salt solution renders the unfertilized Arbacia egg more responsive than normally to the activating influence of hypertonic sea-water. It is significant that in both the muscle and the unfertilized egg the effect is decreased or prevented by the addition of $CaCl_2$ to the pure NaCl

[8] *Cf.* R. S. Lillie: Proc. Soc. Exper. Biol. Med., New York, 1910, vii, 170; This Journal, 1911, xxviii, 197; *cf.* p. 214. The remarkable contact sensitivity induced in voluntary muscle by Na-salts which are Ca-precipitants (J. Loeb: This Journal, 1901, v, 362) is another example of a non-specific sensitization.

solution. And since the most constant feature in the general antagonistic action of Ca is a prevention of certain definite and characteristic surface changes in the protoplasm, as shown by the increase of permeability or other demonstrable alteration of protoplasmic structure (e.g., breakdown of cilia) produced by the pure solution,[9] it seems probable that the primary effect in both the above types of salt sensitization is also a modification of the normal physical and chemical properties of the cell-surface. This surface-change in some manner increases the reactivity of the cell to stimulating or activating influences.

This parallel between the sensitization to chemical stimulation in muscle-cells and the increase of susceptibility to hypertonic sea-water in egg-cells is, however, not complete. Differences are seen both in the reversibility of the effect and in its degree of suppression by $CaCl_2$. The sensitization of a frog's gastrocnemius by pure m/8 NaCl is entirely prevented by the addition of $CaCl_2$ to the solution (e.g., in the proportion 1 Ca to 20 Na), and the sensitized state induced by the pure salt solution is completely and rapidly removed by return to Ringer's solution.[10] On the other hand, in unfertilized Arbacia eggs a definite though usually less well-marked sensitization occurs in the presence of a large proportion of $CaCl_2$ (e.g., in a mixture of 80 vols. 0.54m NaCl plus 20 vols. 0.5m $CaCl_2$), or even in pure isotonic $CaCl_2$ (0.35m); and eggs which have been sensitized in pure 0.54m NaCl can be shown to retain their increased responsiveness to hypertonic sea-water for 24 hours or even longer after the return to normal sea-water. In other words, the increased reactivity induced by the solution is not lost or reversed soon after the return to the normal balanced medium, but persists, apparently so long as the egg remains alive. After remaining for 24 or even 48 hours in sea-water the sensitized eggs show a much more complete response to brief treatment with hypertonic sea-water than do unsensitized control eggs. Some permanent modification, possibly the formation of some specific substance or the production of some structural change essential to normal development, has been induced in the eggs by the exposure to the salt solution.[11]

[9] One of the most satisfactory and convenient test-objects for showing both of these effects of the pure NaCl solution (increase of permeability and breakdown of cilia) and their prevention by $CaCl_2$ is the pigmented trochophore larva of Arenicola cristata at Woods Hole.

[10] Unpublished observations made in the Biological Laboratory of Clark University.

[11] It is interesting to note that recovery from the effect of pure NaCl solution on plant cells (Laminaria) is incomplete when the exposure is prolonged

The question of whether the same kind of modification may be produced in unfertilized eggs by other agents, such as light, mechanical treatment, electrical influences or heat, remains for further experiment to determine.[12] The experiments about to be described show that any well-marked change in the balance of salts in the medium may have this effect, although its degree varies with the character of the solution. Treatment with pure NaCl solution is more effective than with mixtures of NaCl and $CaCl_2$ or with pure $CaCl_2$, but apparently departure in either direction from a balanced condition may induce hypersensitivity of this kind.

Experimental. The following records (tables 1 to 5) of experiments with pure isotonic solutions of NaCl and $CaCl_2$, and with mixtures of the two salts in varying proportions, illustrate the essential nature of the results. A large number of experiments was performed during the period from July to September, and the conditions were not found to vary widely from those described in the tables. There is considerable variation in the susceptibility of normal unsensitized eggs to hypertonic sea-water; but typical exposures of $1\frac{1}{2}$ to 2 hours to the solution used (250 vols. normal sea-water plus 50 vols. 2.5m NaCl) are required to induce the development of a significant proportion of such eggs to free-swimming blastulae. The proportion of eggs that form blastulae with this treatment alone is small, rarely more than 5 to 10 per cent; but eggs that have previously been treated for 5 to 10 minutes with isotonic NaCl solution always yield after exposure to hypertonic sea-water a greatly increased proportion of blastulae, in favorable cases 50 per cent or more, and the optimum duration of the exposure is decreased to 30 or 40 minutes; even 20 minutes is often highly effective with such sensitized eggs, although as a rule less than the optimum. These statements refer to temperatures of 20° to 22°.

beyond a certain point; and that a state of imperfect recovery, i.e., one of more than normal electrical conductivity, may persist unchanged for some days after return from NaCl solution to sea-water (Osterhout: Journ. Gen. Physiol., 1920, iii, 145). This suggests the possibility that altered permeability due to the salt treatment may be an essential factor in the sensitization of Arbacia eggs.

[12] With starfish eggs butyric acid exerts its activating influence from two to three times more rapidly when dissolved in van't Hoff's solution containing 3 to 4 vols. per cent ethyl alcohol than when disssolved in van't Hoff's solution alone; i.e., the alcohol has the effect of sensitizing the eggs to the action of the acid (R. S. Lillie: Journ. Biol. Chem., 1916, xxiv, 233: *cf.* footnote, p. 246). Whether alcohol affects similarly the action of hypertonic sea-water remains to be determined.

On the whole the action of hypertonic sea-water on NaCl-treated eggs is less effective than on eggs in which membranes have previously been formed by a fatty acid or a membrane-forming salt-solution such as pure isotonic KCNS or NaI. This fact indicates that the preliminary stage of the activation, as it occurs in NaCl solutions, is only partial; presumably in order to be complete it should proceed to a point at which the egg separates a fertilization membrane on return to sea-water, but this stage is seldom if ever reached with these solutions.[13] Isotonic solutions of other sodium salts, especially iodide and thiocyanate, may, however, produce the complete effect, and their essential action upon the egg is apparently the same as that of typical membrane-forming agents like weak solutions of butyric acid.

The procedure was as follows. The unfertilized eggs were stirred in sea-water to form a uniform suspension, equal quantities of which were placed in the series of dishes (finger-bowls) corresponding to the several solutions of the series (A, B, C, etc.). When the eggs had settled the sea-water was removed as far as possible by pipette, and to the remaining mass of eggs (*ca.* 2 cc.) a relatively large volume of the solution used (100 cc.) was added. After the lapse of the definite period of exposure (usually 5 or 10 minutes) as much as possible of the solution was poured off and replaced by sea-water; the latter was then changed one or more times after the eggs had settled. After a definite period in sea-water (usually 15 minutes) the eggs were transferred to hypertonic sea-water (usually 250 vols. sea-water plus 50 vols. 2.5m NaCl); at the same time eggs of the control unsensitized lot, which had remained in sea-water, were also placed in hypertonic sea-water. Eggs were returned from the hypertonic sea-water to normal sea-water at intervals of 20, 30, 40 and sometimes 50 minutes; the unsensitized eggs received in addition longer exposures, up to 2 hours. The effects upon the eggs (membrane-formation, cleavage, proportion developing to a free-swimming stage) were later determined.

[13] In the starfish egg a certain minimal duration of exposure to butyric acid of a given concentration is required to cause the separation of the fertilization-membrane; with exposures briefer than this minimum no visible effect is produced on the eggs; but that there has been a partial activating effect is shown by the fact that a second equally brief exposure, applied some time later, causes the eggs to form membranes. This result shows that the condition produced in the egg protoplasm by the acid (new compound formed, or structural change, etc.) may persist unchanged for at least a considerable time without external evidence of its existence (for an instance of this effect *cf.* table 17, p. 292 in my paper in Biol. Bull., 1915, xxviii, 260).

Except in the case of pure 0.54 NaCl no visible external change is produced in the eggs by exposure to the above salt solutions. In the pure isotonic NaCl solution, in addition to some increase of permeability, shown in a slight loss of pigment, there is always a tendency for the eggs to cohere in small clumps or agglutinate loosely; this effect is prevented by a very small addition of $CaCl_2$ (1 mol $CaCl_2$ to 400 NaCl was found sufficient); it is absent in the mixed solutions and in the pure isotonic $CaCl_2$, and indicates a definite change in the physical consistency of the cell-surface, probably associated with the toxic or permeability-increasing action of this solution. The sensitization to hypertonic sea-water appears to be independent of this effect. After the return from the salt solution to sea-water the great majority of the eggs show no further change and respond normally to sperm fertilization, even after an interval of many hours. In the experiments of tables 1 to 3 part of the eggs which had been exposed to each of the salt solutions and returned to sea-water were fertilized with sperm next day, about 20 hours later, and in all cases the majority cleaved and developed. A certain proportion of eggs, however, showed evidence of injury, e.g., defective formation of fertilization-membranes and irregular or delayed cleavage; this was especially the case in eggs which had been exposed to the pure NaCl solution; those exposed to the mixed solutions differed only slightly from the normal. It appears, therefore, that apart from exhibiting an increased responsiveness to hypertonic sea-water the eggs are essentially unchanged in their physiological properties and behavior by the exposure to the salt solutions. This is in agreement with what is observed in other cases of sensitization, where the cells show no change in their normal activities except when exposed to certain special conditions (presence of antigen, chemical stimulation, etc.).

Experiments by Miss Baskervill have shown that varying the time of exposure to pure 0.54m NaCl between 5 and 30 minutes has no appreciable effect on the degree of sensitization; but 45 minutes exposure proved unfavorable to further development, probably because of the general toxic action of the unbalanced solution The essential change produced by the salt solution appears to be completed within the first few minutes of exposure. The return to normal sea-water is not a necessary part of the sensitizing procedure. Eggs transferred from the salt solution directly to hypertonic sea-water show the same increased responsiveness as those placed in this medium after an interval in normal sea-water.

TABLE 1

Series of September 2

PREVIOUS TREATMENT FOR 5 MINUTES WITH FOLLOWING SALT SOLUTIONS	APPROXIMATE PERCENTAGE OF EGGS FORMING SWIMMING BLASTULAE WITH AFTER-TREATMENT WITH HYPERTONIC SEA-WATER FOR THE TIMES INDICATED					
	20 min.	30 min.	40 min.	50 min	70 min.	110 min.
A. Pure 0.54m NaCl	1	4–5	50	30–40		
B. 95 vols. 0.54m NaCl plus 5 vols. 0.5m $CaCl_2$	0	1	*ca.* 5	15–20		
C. 90 vols. 0.54m NaCl plus 10 vols. 0.5m $CaCl_2$	0	*ca.* 10	20–25	25–30		
D. None (control with hypertonic sea-water alone)	0	0	1	4–5	*ca.* 5	*ca.* 5

TABLE 2

Series of September 3

PREVIOUS TREATMENT 10 MINUTES WITH FOLLOWING SALT SOLUTIONS	TIMES IN HYPERTONIC SEA-WATER AND APPROXIMATE PER CENT BLASTULAE				
	20 min.	30 min.	40 min.	60 min.	1 hr. 45 min.
A. 0.54m NaCl	1	2–3	10–15	*ca.* 10	
B. 90 vols. 0.54m NaCl plus 10 vols. 0.5m $CaCl_2$	0	1	1	5–10	
C. 80 vols. 0.54m NaCl plus 20 vols. 0.5m $CaCl_2$	0	1	5–10	1–2	
D. None (control with hypertonic sea-water alone)	0	0	0	1–2	2–3

TABLE 3

Series of September 6

PREVIOUS TREATMENT 10 MINUTES WITH FOLLOWING SALT SOLUTION	TIMES IN HYPERTONIC SEA-WATER AND APPROXIMATE PER CENT BLASTULAE					
	20 min.	30 min.	40 min.	60 min.	1 hr. 30 min.	2hr.
A. 0.54m NaCl	25–30	5–10	5–10	1		
B. 80 vols. 0.54m NaCl plus 20 vols. 0.5m $CaCl_2$	1	1	*ca.* 1	1		
C. 50 vols. 0.54m NaCl plus 50 vols. 0.35m $CaCl_2$	1	1	4–5	1		
D. Pure 0.35m $CaCl_2$	2–3	3–5	5–10	1		
E. None (control with hypertonic sea-water alone)	0	*ca.* 1	2–3	1–2	1	1

(In this series the effective times of exposure to hypertonic sea-water for unsensitized eggs were less than usual.)

In the case of eggs sensitized in pure 0.54m NaCl the optimum time of exposure to hypertonic sea-water of the above concentration (at *ca.* 20°) is typically between 30 and 40 minutes; in my experience (R.S.L.) 40 minutes exposure has usually given somewhat less favorable results than 30 minutes, and at times 20 minutes has proved the most favorable. In general eggs treated with the calcium-containing solutions require for the best results somewhat longer exposures than those treated with the pure NaCl; while normal unsensitized eggs require much longer exposures ((1½ to 2 hours) and a much smaller proportion develop to larval stages.

TABLE 4

Series of September 9

Unfertilized Arbacia eggs were exposed for 10 minutes to the pure 0.54m NaCl solution. From this solution a part was transferred directly to hypertonic sea-water; the remainder to normal sea-water, and from this, after the intervals given, portions were transferred to hypertonic sea-water. The exposures to hypertonic sea-water were 20, 30 and 40 minutes.

INTERVAL IN SEA-WATER BEFORE HYPERTONIC TREATMENT	APPROXIMATE PER CENT OF EGGS FORMING BLASTULAE AFTER EXPOSURE TO HYPERTONIC SEA-WATER FOR FOLLOWING TIMES		
	20 min.	30 min.	40 min.
1. None (direct to hypertonic sea-water)	25–30	10–15	*ca.* 5
2. 2 min	15–20	*ca.* 25	*ca.* 10
3. 4 min	10–15	10–15	5–10
4. 8 min	*ca.* 15	10–15	*ca.* 5
5. 12 min	10–15	10–15	*ca.* 10
6. 18 min	*ca.* 15	10–15	*ca.* 5

Tables 4 and 5 summarize the results of experiments in which the eggs, after treatment for 10 minutes with pure 0.54m NaCl, were exposed to hypertonic sea-water for 20, 30 and 40 minutes after a variable interval in normal sea-water.

It will be noted that varying the interval between the salt treatment and the exposure to hypertonic sea-water between 0 and 30 minutes has no significant influence on the proportion of eggs developing to the blastula stage. The response of the sensitized eggs to the treatment with hypertonic sea-water shows considerable irregular variation both in the proportion of eggs reaching advanced states and in the optimum times of exposure. On September 9th the best results were obtained after 20 and 30 minutes in hypertonic sea-water, with a well-marked de-

cline at 40 minutes. On September 12th an exposure of 20 minutes had little effect, while 30 and 40 minutes were about equally effective. In a large number of other experiments by Miss Baskervill the optimum exposure was usually found to lie between 30 and 45 minutes. Exposures of 60 minutes and longer were less favorable. With unsensitized eggs the optimum exposures are two or three times longer, as already pointed out.

In a similar series of experiments on September 8th part of the eggs were treated for 10 minutes with pure 0.54m NaCl, and part with a mixture of 90 vols. 0.54m NaCl plus 10 vols. 0.5m $CaCl_2$; then, after intervals in sea-water varying from 0 to 8 minutes, they were exposed to

TABLE 5

Series of September 12 and 13

In both series eggs were exposed to 0.54m NaCl for 10 minutes. Otherwise like the series of September 9, except that the intervals between the successive transfers to sea-water were longer.

INTERVAL IN SEA-WATER	TIME IN HYPERTONIC SEA-WATER AND APPROXIMATE PER CENT OF BLASTULAE					
	20 min.		30 min.		40 min.	
	September 12	September 13	September 12	September 13	September 12	September 13
1. None (direct to hypertonic)...	1	3–5	*ca.* 5	10–15	*ca.* 5	3–5
2. 5 min.....	1	3–5	*ca.* 5	15–20	*ca.* 5	15–20
3. 10 min.....	1	3–5	4–5	20–25	4–5	*ca.* 20
4. 20 min.....	0	4–5	4–5	15–20	4–5	15–20
5. 30 min.....	0	*ca.* 4–5	3–5	*ca.* 20	4–5	*ca.* 20
6. Control (no salt treatment)....	0	0	0	1	1	1

hypertonic sea-water for the two periods of 20 and 30 minutes. The difference between the pure and the calcium-containing solutions was striking; with the former solution the percentages of blastulae ranged from 3 to 5 with the 20 minutes after-treatment, and from 5 to 20 with the 30 minutes; while with the calcium-containing solution almost no eggs formed blastulae with the 20 minutes exposure and not more than 1 per cent with the 30 minutes. The optimum duration of after-exposure was probably not reached in this case. The anti-sensitizing influence of the calcium was especially well marked in this experiment.

Experiments with pure and calcium-containing solutions of several other sodium salts ($NaNO_3$, $NaSO_4$, Na-citrate) gave results of the same general kind as with NaCl. The sensitizing action was distinct and not

essentially different from that in NaCl. No special peculiarities of action were observed that could be related to the valence or other varying properties of the ions, but the number of experiments was relatively small. The best results were obtained with 5 to 10 minutes in the salt solution, followed by 45 minutes in hypertonic sea-water of the above composition. Exposures of 1 hour to hypertonic sea-water were less favorable.

The experiments of tables 4 and 5 have shown that varying the interval between the two treatments has no significant influence on the proportion of eggs developing to larval stages. Evidently the physiological effect produced by the salt solution is not reversed by the return to sea-water. Even after 24 hours or longer in sea-water the eggs retain apparently unimpaired the increased responsiveness to hypertonic sea-water.

Further experiment has shown that the physiological effects produced by the isotonic salt solution and by the hypertonic sea-water are different in kind, and that the order of the two treatments cannot be reversed. Eggs which have been exposed to hypertonic sea-water for 30 to 40 minutes without previous treatment with salt solution cannot afterwards be induced to develop by treatment with pure isotonic NaCl solution. The only evident effect of the pure salt solution upon eggs thus treated with hypertonic sea-water and returned for an interval to normal sea-water is an increased tendency to cytolysis, and the proportion forming larvae —in any case small with so brief an exposure to hypertonic sea-water— is distinctly decreased. The after-exposure to the salt solution is injurious and gives no increased impulse to development. There is no evidence that it tends to complete the partial activation resulting from the brief exposure to hypertonic sea-water.

The following is a summary of a typical experiment (Sept. 13, 1920). Unfertilized Arbacia eggs were divided into three lots, A, B and C. The eggs of lot A were placed for 10 minutes in 0.54m NaCl and replaced in sea-water. Next day, after 21 hours in sea-water, they were exposed to hypertonic sea-water for 20, 30 and 40 minutes. Those of lot B were first exposed to hypertonic sea-water for 20, 30 and 40 minutes and left in normal sea-water for 21 hours; they were then exposed for 10 minutes to 0.54m NaCl and returned to sea-water. Those of lot C were left untreated in normal sea-water over night and exposed to hypertonic sea-water for 20, 30 and 40 minutes at the same time as lot A. The results were definite and characteristic. Of lot A the percentages of eggs forming blastulae with the three exposures, 20, 30 and 40 minutes, were, respectively, less than 1 per cent, *ca.* 3 per cent, and between 5 and 10

per cent. Of lot B only a few scattered eggs formed blastulae, fewer than in lot C of which less than 1 per cent developed to this stage, even with 40 minutes exposure.

Three other similar series gave the same general result. On September 16th unfertilized eggs which had been left in sea-water for 24 hours after a 10 minutes' exposure to isotonic NaCl solution and were then treated for 40 minutes with hypertonic sea-water, gave 4 to 5 per cent of blastulae; even after 48 hours in sea-water almost the same proportion of blastulae was obtained. The series of September 18th was still more striking; after 24 hours in sea-water the salt-treated eggs gave 10 to 20 per cent of blastulae with exposures of 30 and 40 minutes to hypertonic sea-water; and eggs left for 48 hours in sea-water before the hypertonic treatment again yielded a considerable though smaller proportion of blastulae. On the other hand, when the freshly removed eggs were treated with hypertonic sea-water, left in sea-water for 24 hours, and then exposed to NaCl solution, the only visible effects of the salt treatment were agglutination and increased breakdown. Not the slightest evidence of improved conditions of development was seen in any experiment.[14]

Theoretical. The physiological change induced by the salt solution, involving a greatly increased responsiveness to the activating influence of the hypertonic sea-water, has been described above as a "sensitization;" but such a characterization throws little light on its real nature beyond implying resemblance to the other phenomena of sensitization in living cells. There is little doubt that highly specific forms of sensitization, such as the anaphylactic sensitization of smooth muscle-cells in guinea pigs and other mammals, are referable to the presence in the irritable cell of newly formed specific compounds whose chemical interaction with the antigenic substance gives rise in some manner to strong stimulation.[15] These compounds are the products of a specifically

[14] In the case of Strongylocentrotus Loeb found that eggs subjected first to a somewhat prolonged treatment with hypertonic sea-water (about twice the optimum for eggs with previously separated membranes), and afterwards exposed to the membrane-forming agent (butyric acid), developed in a larger proportion of cases than with the hypertonic treatment alone (J. Loeb: Artificial parthenogenesis and fertilization, p. 110). In this case the order of the two treatments can apparently be reversed without changing the essential character of the results. The time relations are, however, different in the two cases.

[15] The direct condition of stimulation may be a precipitating or other structure-altering effect caused by interaction of the anti-body in the cell-surface with the antigen in the external medium. There are indications that the stimu-

altered metabolism, and it is probable that they are situated largely in the surface-layer of the cell (though they may not be confined to this region); this is indicated by the promptitude with which the sensitized muscle contracts when brought into contact with serum containing the antigen, and also by the phenomena of passive sensitization, which indicate that the circulating antibody is attracted to (e.g., adsorbed) or fixed by the cell surface. In the case of the egg also we may assume that some stable specific substance, which is in some way necessary for development, is formed, or that some definite structural change is produced, as a result of altered metabolism due to the treatment with the salt solution. It is significant that the altered condition is not reversed by the return to the normal medium, as would presumably be the case if (e.g.) simple colloidal changes dependent on altered equilibrium between the salts of the medium and the cell-colloids were concerned. The eggs show no outward evidence of structural change (such as membrane-formation) and they respond normally to sperm-fertilization. Some permanent change in the chemical composition of the internal cell-protoplasm is indicated.

The determining condition of this change is almost certainly some action upon the protoplasmic surface-layer, since it is produced not only by pure NaCl solutions, but also by approximately balanced solutions of NaCl and $CaCl_2$; during the brief period of exposure (5 to 10 minutes) to the isotonic solution there can be little if any penetration of salt into the interior of the egg. Apparently, also, since under these conditions there is no sign of membrane-formation, the separation of a visible fertilization-membrane is an incidental rather than an essential feature of the sensitization-process. The conditions in the Asterias egg, as well as in Arbacia, indicate that a membrane is separated only after the earlier part of the activation-process has reached a certain stage of completion. We may infer that the above salt solutions are less effective than fatty acids or isotonic KCNS or NaI solutions in increasing the responsiveness to the hypertonic treatment because of the incomplete nature of the reaction which they induce. The effect is of the same kind, only partial.

Ultimately the above permanent increase of susceptibility to hypertonic sea-water must be attributed to some definite and specific change

lating reaction and the precipitin reaction are chemically identical, although occurring in different situations and under different conditions. *Cf.* the recent Croonian lecture of H. H. Dale on Anaphylaxis, Proc. Roy. Soc., B., 1920, xci, 126.

in the processes of constructive metabolism occurring in the cell-interior. Apparently this specific metabolic change follows as a more or less direct consequence of altered surface-conditions, just as in many other cells extensive changes of metabolism, e.g., of oxidation-rate, involving the whole active protoplasm, may be initiated by some external process affecting directly only the cell-surface, e.g., the response of a muscle-cell to electrical or mechanical stimulation. The change produced by the salt solution in the physiological properties and reactivity of the egg-system may be the result of altered chemical composition, or of altered structure, or of both combined. It seems probable that some relatively simple initial chemical effect results from the surface-action of the salt solution, and that this leads secondarily to an altered sequence of metabolic processes in the cell-interior. This primary chemical effect might conceivably be a change in the rate or character of the oxidations. According to Loeb and Wasteneys, however, the oxygen consumption in freshly fertilized Arbacia eggs is not changed by isotonic NaCl solution.[16] In the Strongylocentrotus egg, on the other hand, there is a marked increase, according to Warburg.[17] The conditions in the unfertilized Arbacia egg do not appear to have been investigated.

A greater degree of certainty seems to prevail regarding the general nature of the physico-chemical change first produced by the salt solution. Pure isotonic NaCl solution causes an increase of ionic permeability in the plasma-membranes of many and perhaps all cells; to this change must correspond a change of electrical surface polarization. The primary effect of the electric current on living cells is, however (according to Nernst's theory of electrical stimulation[18]), to produce changes of electrical polarization at the semi-permeable surfaces separating the protoplasm from the external medium; evidently this implies—since the current causes stimulation and other changes of activity—that variations in the potential difference across the cell-surface have a controlling influence on the internal metabolism of the cell. It seems probable that the action of salt solutions upon unfertilized eggs belongs in this general

[16] J. Loeb and H. Wasteneys: Biochem. Zeitschr., 1910, xxviii, 340.

[17] O. Warburg: Zeitschr. physiol. Chem., 1910, lxvi, 305. Warburg finds also that various membrane-forming agents increase the oxygen-consumption of unfertilized Strongylocentrotus eggs to a point about equal to that shown by normally fertilized eggs. It thus appears probable that a partial effect, such as that produced in Arbacia eggs by NaCl solutions, would have a similar accelerating influence on oxidations.

[18] Nernst: Arch. f. d. gesammt. Physiol., 1908, cxxii, 275.

class of effects. In any case variations in the salt content of the external medium must influence the conditions of electrical polarization at the cell-surface.

SUMMARY

1. Exposure of unfertilized Arbacia eggs to pure isotonic solutions of NaCl for 5 to 10 minutes (at 20° to 22°) greatly increases the responsiveness to a subsequent brief treatment (20 to 45 minutes) with hypertonic sea-water. Other neutral Na salts (nitrate, sulphate, citrate) have a similar effect.

2. This sensitizing effect of the salt solution may be decreased although not prevented by the addition of $CaCl_2$ to the pure NaCl solution. Apparently it results from any sufficient change in the salt balance. Even pure isotonic $CaCl_2$ solution produces the effect.

3. The sensitized eggs show no other evidence of alteration. They do not form fertilization-membranes and may remain living and fertilizable for 24 hours or more. The sensitized condition is not appreciably reversed during the stay in sea-water, but may persist for so long as 48 hours.

IS CATALASE A MEASURE OF METABOLIC ACTIVITY?

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When one considers the large number of investigations on catalase, the net result seems exceedingly discouraging, for in all frankness it must be admitted that the functions of this most widely distributed enzyme are still practically unknown. It is therefore to be particularly regretted that this unsolved problem of the significance of the catalase should be further perplexed by a theoretical superstructure of questionable merit. A score of years or more ago Spitzer (13) conceived the idea of a possible connection between the catalase and functional activity. In recent years a similar idea has been greatly elaborated by Burge (4) who offered this as an easy and ready explanation of nearly every problem in the field of biology. It would require too much space to enumerate the various biological phenomena which Burge has brought under the simple hypothesis of the direct relationship between the amount of catalase and metabolic activity.

In his earlier work Burge compared the catalase activity of samples of blood obtained from an animal under different experimental conditions. Where the experiments have been repeated by others, Burge's hypothesis was not corroborated by the new results. Thus, Becht (2), studying the catalase of blood pointed out the important fact that even in a normal animal this is subject to very wide variations (about 1000 per cent!). He demonstrated, furthermore, that the catalytic power of any sample of blood depends upon the number of red cells, a fact which had been recognized already by Löb and Mulzer (8). Becht failed to substantiate the results on the effect of anesthetics which led Burge to formulate the opinion that the lowering of the animal's metabolic activity is accompanied by a parallel lowering in the catalase content of the blood. Reiman and Becker (12) were likewise unable to confirm Burge's results on the effect of anesthesia.

Stehle (14) has gone over several of Burge's experiments which have furnished the arguments in favor of the hypothesis that the amount of catalase is proportional to the oxidative processes in the organism. Stehle, however, failed to corroborate Burge's results in that he either found no change in the catalase content of the blood or only a small increase under the conditions of the experiments. Stehle was also led to believe by the evidence he obtained that the variations in the catalase activity of the blood were due to fluctuations in the number of the red blood cells in the sample.

In recent work Burge has departed from the original technique of experimentation, but is still adhering to his interpretation that catalase is a measure of the vigor and intensity of life processes. Experimenting with potato beetles in different stages of development, he brings forth new evidence in support of his contention showing that as the metabolic activity changes during the life cycle of the animal, the catalase content also increases. Measuring the catalase activity by the number of cubic centimeters of oxygen set free from hydrogen peroxide[1] by 0.5 gram of substance at different stages in the development, from the unfertilized egg to the adult insect, he notes a hundred-fold increase (18 to 1800 cc.). The low rate of oxidation in the unfertilized ovum Burge attributes to its low catalase content. The increase in the oxidative processes in the ovum following fertilization he attributes to an increase in the catalase through augmented production of the enzyme occasioned by the stimulating action of the spermatozoön. From these experiments, in a characteristic manner, Burge makes the deduction that "similarly the increase in the respiratory metabolism or oxidation in youth and its decrease in old age is attributed to an increase in the catalase in the young and to its decrease in the aged." Similar results are also reported with mice of different ages.

It cannot be denied that the catalase content of different parts of an organ, of different functional value, may sometimes reveal quantitative differences. In experiments conducted in conjunction with V. E. Levine (9) on the cortex, medulla and papillary region of kidneys from various animals we found invariably that the catalase content was greatest in the cortex and smallest in the papillary portion. We did not, however, feel justified in ascribing these differences to a metabolic gradient, though we were able to show, by experiments on perfused kidneys, that the variations in catalase are not due to differences in blood content.

[1]Actual amount of hydrogen peroxide not indicated.

Utilizing Edwards' (6) observation on the physiological differences in segments of the Limulus heart, viz., that the contractility of different segments diminishes from the anterior segment backward, the author studied their catalase content but failed to find any parallelism with the physiological observations. Alvarez and Starkweather (1) working with segments from the intestinal tract were able to show that the catalase content follows the functional gradient observed along the intestinal tract. They, therefore, seem to have accepted Burge's hypothesis that physiological function is paralleled by catalase activity.

To put the theory to a direct test I undertook a series of experiments with frogs which were kept at widely different temperatures. It is a definitely established fact that the metabolic activity of poikilothermic animals varies with the surrounding temperature. This was shown by Vernon (15) for frogs as far back as 1894, when he demonstrated that the evolution of carbon dioxide varies with the body temperature of the animals. Cronheim (5), experimenting with fish, found the same relationship between metabolism and surrounding temperature. Thus, at 8°C. carp consumed 600 cc. of oxygen per kilogram and per 24 hours, at 20°C. 2250 cc.; and at 25°C. as much as 2600 cc. In other words, the metabolism increased more than fourfold while the temperature increased about three times. Brunow (3) likewise has shown in his experiments with fresh-water crabs that the carbon dioxide production and the oxygen consumption depend on the temperature of the medium. Within the limits of from 5°C. to 21°C. the metabolism is an exponential function of the temperature, i.e., it increases more rapidly than the temperature. For the range of temperature variations from 5° to 20°C. he found a fourfold increase in the metabolic activity.

Bearing in mind these fundamental facts it seemed to me that they offered an exceptional opportunity for putting Burge's catalase theory to a crucial test. If catalase is a measure of metabolic activity, then evidently the catalase content of frogs kept at widely different temperatures should likewise show large differences.

Before passing on to a description of the experiments and the results obtained, it is necessary to make a digression and discuss some important facts pertaining to the catalase reaction itself which are essential in the interpretation of the results. In a paper on the chemistry of the catalase reaction now in press, the author (10) has shown that this is of the type of a balanced reaction, and that the maximum activity of the catalase is obtained when about 70 per cent of the amount of hydrogen peroxide has undergone decomposition. Furthermore, it has been

shown that an excess of the peroxide causes a dropping off in the amount of oxygen set free by a certain quantity of catalase. However, within the limits of 60 to 100 per cent decomposition the amount of oxygen liberated is a linear function of the quantity of catalase. Theoretically, therefore, it is best to adjust the amount of catalase so that 70 per cent of the hydrogen peroxide will be decomposed. In practice, of course, this is not necessary and it is sufficient to find a suitable quantity of catalase to effect the decomposition of from 60 to 100 per cent of the hydrogen peroxide since this will allow to find the quantity required for 70 per cent decomposition by interpolation. Frequently the calculated amount of catalase has been verified by an experiment but since the determined quantity and the calculated value were always found to check very closely this was not carried out in each experiment.

Another important point to be considered is with regard to the basis which should be assumed in making the comparison of the catalase activity of different preparations. The method generally followed in experiments on catalase has been to determine the amount of oxygen liberated from a certain quantity of hydrogen peroxide by similar amounts of the substance under investigation. The catalase activity is assumed to be proportional to the quantity of oxygen produced. It would take us too far afield to show why this reasoning is theoretically erroneous. Osterhout (11) discussed this question at some length and his article may be consulted in this connection. Suffice it to say here that in attempting a comparison of the activity of different samples of biological material it is necessary to find the respective quantities of material which will effect the same result, i.e., will set free the same number of cubic centimeters of oxygen from a given amount of hydrogen peroxide. In all our experiments the attempt has been made to bring about a 70 per cent decomposition with the same initial quantity of peroxide. The relative activity of the catalase preparations is, therefore, compared on the basis of the respective amounts of the enzyme accomplishing the same work. The catalase activity, of course, varies inversely as the quantities producing the same effect. For instance, if 1 cc. of one preparation and 2 cc. of another give the same quantitative result, the former has exactly twice as much catalase as the latter.

Still another question which must be considered is how long a catalase experiment should be allowed to run. In most experiments on the catalases an arbitrary period has been accepted (usually 10 minutes). Such a procedure is entirely indefensible. Of course, it might be argued that since only comparative values are sought it matters little whether

the reaction is fully or partly completed. This argument might have some force if the tacit assumption that the activity manifested by the catalase is a function of time were true. This, however, is not the case. From the results recorded in a subsequent portion of this paper it will be seen that the reaction is only about 50 per cent complete within 10 minutes. In our experiments, however, the ratio between the catalase and the hydrogen peroxide has been so arranged that the reaction follows the isotherm of a bimolecular equation, and therefore the quantities of oxygen set free in the same periods of time are strictly comparable. When such a condition is not definitely established the comparison on the basis of equal time intervals may have perhaps value from a qualitative but certainly not a quantitative point of view. Although a certain amount of catalase can decompose a definite amount of hydrogen peroxide the final result depends upon two conditions. There must be an optimum hydrogen ion concentration, or at any rate the pH of the reaction mixture should not fall below 6.0. Burge mentions that he uses neutral hydrogen peroxide but does not specify whether this neutrality means a pH of 7.0, or that titration to the zone of neutrality with some indicator like congo red (the indicator which has commonly been used in catalase investigations) is relied upon, in which case the hydrogen peroxide from the point of view of its hydrogen ion concentration is still acid. Again, when the hydrogen peroxide is much in excess of the quantity of catalase with which it is made to react, the amout of oxygen liberated diminishes and the more so the greater the excess of peroxide. In the experiments with the potato beetle material, already referred to, Burge finds that half a gram of unfertilized eggs gives off only one-hundredth the amount of oxygen set free by a half gram of pupae material. Judging by the quantity of oxygen produced Burge must have employed no less than 150 cc. of peroxide ("Dioxogen"). From my own experience with catalases I can say definitely that if the same quantity of peroxide had been used with the egg material—and according to the description of the technique in the papers mentioned this must have been the case—the catalase activity of the latter would be reduced to perhaps one-tenth of its actual strength measured under proper conditions. While this does not imply that there may not be a difference in the catalase strength of the unfertilized beetle eggs and of the pupae, the differences as found by Burge are grossly exaggerated owing to the defect of his experimental procedure.

To come back, however, to the original question as to how long the catalase experiment should be allowed to run. The answer is simple—until the reaction comes to a stop. It is possible, of course, that the reaction is over in 10 minutes provided the *excess* of the catalase is sufficiently large. In that case, however, the excess remains undetermined. In my own experience in standardizing catalase preparations it has not infrequently happened that after repeated trials the quantity just sufficient to bring about complete decomposition of the hydrogen peroxide was perhaps one-tenth of the amount first tested. On the other hand, if the reaction is not complete within the 10 minutes, i.e., if only a fraction of the hydrogen peroxide has undergone decomposition, one is also at a loss inasmuch as the reaction velocities vary directly as the catalase quantities. The tendency will therefore again be for the differences to become exaggerated.

With these preliminary remarks we may now pass to a consideration of the experimental data. Frogs of uniform size and weight were kept for 48 to 72 hours under different temperatures with ample provision for moisture. By placing the receptacle with the frog directly by the ice in a cold box a temperature of 4 to 5°C. was obtained, while the temperature of 30°C. was maintained with a thermostat. The animals were left at these different temperatures for a long enough time to allow perfect reajustment to the changed environmental condition. To forestall any possible criticism that the failure to discover quantitative differences in the catalase content of some particular organ neither proves nor disproves the contention that the metabolic rate is regulated by the amount of catalase present, being due to a shifting of the catalase within the organism, the whole frog was triturated with Berkshire sand in a mortar to a very fine pulp. The ground material was extracted with water saturated with chloroform and the catalase determinations made on aliquot portions of this water extract. In the second series of experiments the frogs were ground without the skins inasmuch as it has been found practically impossible to triturate these. After standing over night in the ice chest the material was centrifuged, the solid residue again extracted with chloroform water, and the total extract made up to a definite volume. The extract was then filtered through a hardened paper. The experiments were performed at 20°C., the volume of the reacting mixture being always 50 cc. The hydrogen ion concentration was directly controlled close to the optimum point (6.9 to 7.3 pH) and the oxygen value of the hydrogen peroxide determined by titration with $KMnO_4$. The results given in this paper have been corrected for temperature and pressure.

Series 1. Frog 1 was exposed to a temperature of 4 to 5°C. for 48 hours. Killed by pithing; total extract was 320 cc. One cubic centimeter of this extract reacting with a quantity of hydrogen peroxide equivalent to 50 cc. of oxygen set free 44.6 cc. In other words, 89.2 per cent of the hydrogen peroxide was decomposed. From this experiment it can be calculated that 0.785 cc. of the extract would effect a 70 per cent decomposition (i.e., would set free 35 cc. of oxygen).

Frog 2. This frog was kept at 30°C. for 48 hours. Prepared in the usual way; the extract was made up to 300 cc. One cubic centimeter of this extract reacting with a similar quantity of hydrogen peroxide produced only 30.8 cc. of oxygen (61.6 per cent of hydrogen peroxide decomposed). From this it may be calculated that 1.136 cc. would be required for a 70 per cent decomposition. The final volumes of the two extracts not being exactly the same, the relative catalase strengths of the two preparations can be regarded only approximately as 1:0.7 (reciprocals of the quantities producing the same amount of work). We may also determine the total catalase content of each extract. Since 0.785 cc. of the cold frog extract can set free 35 cc. of oxygen, the entire extract would liberate 14.27 liters of oxygen. This frog weighed 24 grams, therefore, a gram of the frog substance can set free 595 cc. of oxygen (i.e., it could decompose about 50 cc. of "Dioxogen" hydrogen peroxide). Applying the same calculation to the 30° frog, the total extract could set free 9.1 liters of oxygen ($\frac{300 \times 35}{1.136} = 9100$ cc.). This frog weighed 22 grams; hence, the catalase activity of a gram of frog substance is equal to 414 cc. of oxygen (would decompose approximately 36 cc. of "Dioxogen"). Thus, the frog with the much greater metabolic activity possessed only about 70 per cent of the catalase content of the cold frog. It is possible that at 30° the catalase had actually undergone decomposition within the frog's organism. Such a possibility is suggested by the experiments with frogs kept at 20°C. If this is actually the case, the fact would be most astonishing because a catalase preparation is not destroyed by heat below 60°C. and according to Issajew (7) 40°C. is the optimum. However, it may very well be that the overstimulation of the catabolic processes in the frog by a high temperature involves also the destruction of catalase.

Frog 3. The third frog of this series was kept at 20°C. and has been worked over precisely as the other two frogs. The extract was made up to 300 cc. Using hydrogen peroxide enough to yield 100 cc. of oxygen, it was found that 1.7 cc. of the extract produced 84.8 per cent decompo-

sition. On this basis we may calculate that 1.4 cc. would be required to effect a 70 per cent decomposition. An experiment was performed with 1.35 cc. of the extract with which 66.1 per cent decomposition was brought about. On the basis of this experiment 1.43 cc. would be necessary for a 70 per cent decomposition. It is clear, therefore, that the method of calculation is reliable within 2 per cent. Taking the average of the two calculated amounts, 1.415 cc. of extract can yield 70 cc. of oxygen, or the entire extract would liberate 14.91 liters $\left(\frac{300 \times 70}{1.415}\right)$. The frog weighed 24 grams so that the catalytic capacity of 1 gram of its substance is 621 cc. of oxygen. Considering that the metabolism at 20°C. was probably four times as great as that of the frog kept at 4°C. the difference of less than 5 per cent in the catalase content of the two frogs (621–595) is practically neglible.

Series 2. These experiments were made with two frogs kept for 72 hours at 5° and 20°C. respectively. The animals were prepared in the same way as in the previous series except that the skins were not used. The frogs were each ground up with 8 grams of sand and extracted three times. The final volume of the extract was 300 cc. The experiments were performed at 20°C. using enough hydrogen peroxide to furnish 100 cc. of oxygen. The pH of the mixture was 6.9 to 7.0.

Frog 4. Live weight—18.5 grams; skinned—16.8 grams; temperature 20°C.

Frog 5. Live weight—17.0 grams; skinned—15.0 grams; temperature 5°C.

In each case 2 cc. of the extract produced 81 per cent decomposition of the hydrogen peroxide. The results recorded in the table show a correspondence between the two catalase preparations which could not be surpassed by a duplicate determination with the same sample. In either case the decomposition follows strictly the equation for a bimolecular reaction, the average velocity coefficient in both instances being 0.00075.

From the results presented in the table it can be calculated that frog 4 can set free 12.18 liters of oxygen, or 715 cc. per gram of substance, while frog 5 has a capacity of 12.15 liters, or 810 cc. per gram.

The exposure to widely different temperatures, which it is well known can effect a change in the metabolic rate of from 300 to 400 per cent, obviously had no influence on the catalase content of the frogs. The conclusion is therefore unavoidable that whatever the function of catalase in the organism may be, it is certain that it is not a measure of metabolic activity.

TABLE 1

Cubic centimeter of oxygen evolved by 2 cc. of extract (H_2O_2 = 100 cc. O_2)

TIME IN MINUTES	FROG 4 (AT 20°C.)	FROG 5 (AT 5°C.)
5	27.4	28.2
10	40.3	41.1
15	50.2	51.0
20	58.5	59.1
25	65.6	65.4
30	70.0	69.8
35	73.3	73.3
40	76.1	75.9
45	77.9	77.7
50	79.3	79.3
55	80.3	80.2
60	81.2	81.0

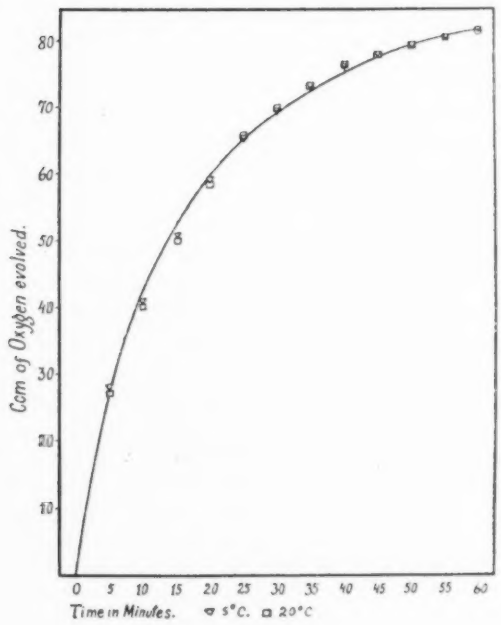

Fig. 1

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INFLUENCE OF GLANDS WITH INTERNAL SECRETION ON THE RESPIRATORY EXCHANGE

II. Effect of Suprarenal Insufficiency (by Removal or by Freezing) in Rabbits[1]

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The fact that animals which survive double suprarenalectomy for a week or more, frequently lose weight rapidly was pointed out by the earliest observers (1), (2), (3), (4). This loss of weight is largely due to the rapid disappearance of fat. Likewise, Porges (5), Schwartz (6) and many others have shown that suprarenalectomy causes a rapid disappearance of glycogen from the liver and a progressive fall in the blood sugar in dogs and rats. Notwithstanding these striking manifestations of suprarenalectomy their possible relation to alterations in the rate of metabolism seems to have attracted very little attention.

Golyakowski (7) in 1899 published a preliminary report on his observations on twelve dogs in which most of the blood supply to the suprarenals was blocked by mass ligation (the exact nature of this operation is not given). He reported that three died within 3 days, one lived 28 days and eight survived longer than 6 weeks. In those animals which survived over 6 weeks, he noted a rise up to 30 per cent in heat production and CO_2 output within the first 10 days, followed by a drop nearly to normal, then a second rise as high as 50 per cent between the 2nd and 4th weeks falling to or even below normal about the 6th or 7th week. Heat production and heat loss were parallel, from which he concluded the heat regulating mechanism was intact. While emphasizing the increase in heat and CO_2 production, he also states there was little or no increase in O_2 consumption above the normal. He realized the discrepancy between the CO_2 and O_2 values, but he

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explained the low O_2 intake by assuming that the extra necessary O_2 was derived from the tissues. On account of this statement together with the absence of any experimental data or descriptions of methods used one cannot attach the importance to his conclusions to which they would otherwise be entitled. We have been unable to find any other references to work on the effect of removal of the suprarenal glands on the respiratory exchange except that reported by Aub (8) and his co-workers. They reported briefly the effects of suprarenalectomy on the respiratory exchange in three cats and found "there was a slight rise in the metabolic rate for some hours, and 48 hours after operation a sharp fall to about 25 per cent below normal. This remained until the animals were sacrificed 5 days after operation." At the same meeting we reported strikingly different observations on the effects of suprarenalectomy on the respiratory exchange in rabbits. It is, therefore, the purpose of this paper to report in some detail the types of modification observed in the respiratory exchange.

Our experience with suprarenalectomized cats has been strikingly similar to that of Aub, except that the deterioration of the animals as measured by the metabolic rate and duration of life was even more rapid. Cats, dogs and guinea pigs usually survive the operation of double suprarenalectomy, whether performed at one or two sittings, only a few days. This fact limits the study of the metabolic changes to the immediate or acute effects of suprarenal insufficiency. White rats withstand double suprarenalectomy too well. The wide variation in duration of life following double suprarenalectomy in rabbits makes it possible easily to obtain metabolic studies on a series of animals surviving the operation from a few days on to indefinite survival. The varying lengths of time which rabbits survive extirpation of the suprarenals is believed to depend upon the presence of varying amounts of accessory cortical tissue, lying for the most part in the neighborhood of the suprarenal glands or along the path of migration of the sex glands. Paradoxical as it may seem the presence of accessory cortical tissue makes it possible to obtain more graded series of incomplete suprarenalectomies than can be obtained by attempting to leave intact varying amounts of the main glands. This is due to the impossibility of preserving unimpaired the blood supply of the unremoved portion. The importance of keeping this in mind becomes even greater if we realize how small a fragment of functionally active suprarenal cortex suffices to maintain the animal in apparently normal health. It was for these reasons that the rabbit was selected as being the most suitable animal for this study.

Methods. The respiratory exchange was determined with the Haldane apparatus, modified as described in a previous paper (9). Rabbits were kept under comparatively uniform conditions. Their diet consisted of alfalfa hay, oats and carrots. They were last fed 15 or 16 hours before the respiratory exchange was determined. The gaseous exchange was determined for a period of 2 hours in each instance. Forty-two rabbits have been used. Control metabolic rates were obtained for a period of several weeks, sometimes before removal of the right suprarenal and sometimes after its removal. No significant change in the metabolic rate or departure from the normal behavior of the animal has been noted following the removal of one suprarenal except in one instance (rabbit 216). In a small group (seventeen) the attempt was made to destroy the function of the cortex of one or both glands by freezing and at the same time to leave the medulla intact. This was done by isolating the gland with as little injury to its blood vessels and nerves as possible and freezing for 30 to 45 seconds with a spray of ethyl chloride. In several instances we succeeded in severely injuring most of the cortex except that portion immediately adjacent or within the medulla, while at the same time preserving most of the medulla with its blood supply intact, as determined later histologically. The method of separating medulla from cortex by freezing, while highly efficient and simple, could not be utilized in effecting a complete separation in rabbits because of the anatomical relationships of cortex and medulla. Likewise, it is not readily applicable to the right suprarenal in this animal because of the relation of the gland to the vena cava. Marked suprarenal insufficiency has been obtained in a number of instances by this method without destroying the medulla as determined by the physiological behavior of the animal and histological examination.

Presentation of data. The following eight protocols have been selected from the 42 experiments of this series as types of the alterations observed in the respiratory exchange. These types may be divided more or less arbitrarily and for convenience of presentation into 3 groups, depending on the completeness of removal and the time of survival, namely:

I. Those that live indefinitely after removal or injury of the suprarenals with no appreciable alteration in the respiratory exchange.

II. Those that show an increase in the respiratory exchange followed by a fall, to, or below normal, whether dying within 2 or 3 weeks or living on indefinitely.

III. Those that show a fall of the metabolic rate beginning within 48 hours after the removal or injury of the glands and continuing to death.

In addition to these groups representing suprarenal injury or removal, it has seemed advisable to add for the sake of comparison one protocol showing the effect of desiccated thyroid feeding in a "normal" rabbit.

GROUP I. *Protocol I.* Rabbit 232; female.

November 26, 1920. Under ether removed right suprarenal completely

December 5, 1920. Wound healed, stitches removed, weight 2810 grams.

December 22, 1920. Began metabolic studies

January 8–11, 1921. Gave total of 0.4 gram potassium iodid by mouth

January 24, 1921. Gave birth to young—destroyed

February 25, 1921. Injected 0.5 gram cholesterol (from gallstones) intraperitoneally

March 22, 1921. Under ether removed left suprarenal completely

March 31, 1921. Wound healed

April 19, 1921. Died this morning, necropsy, body still warm, pregnant near term, 6 embryos; ovaries very large, pale yellow, five distinct corpora lutea; thymus present; thyroids vascular; parathyroids vascular; liver large, pale grayish yellow brown, nutmeg appearance, fatty. Whitish subcapsular deposits probably cholesterol; kidneys enlarged, cortex swollen, grayish and possibly fatty; heart moderate hypertrophy; right and left suprarenal glands absent; no accessories found on careful search; spleen normal in size; solitary gastric ulcer about 1 cm. from pylorus; death probably from acute suprarenal insufficiency developing late in, and in relation to pregnancy with renal and hepatic lesions resembling those of toxaemia of pregnancy.

Protocol II. Rabbit 255, female

March 2, 1921. Began metabolic studies

March 22, 1921. Under ether the right and left suprarenals were exposed through usual incisions for removal and frozen with ethyl chloride spray; the blood vessels were not divided

April 1, 1921. Wounds healed and stitches removed

April 15, 1921. Healthy, strong, killed, immediate necropsy. All viscera appear normal, except suprarenals; right suprarenal easily isolated, upper portion firm, yellow brown, while portion on inferior cava shows soft, vascular, pale yellow, normal-looking cortical mass approximately 3×3 mm.; left suprarenal readily enucleated, upper portion dry, firm, yellow brown with grayish tint, lower portion of gland adjacent renal vein is soft vascular and measures approximately 2×2 mm.; no accessory suprarenals found; both ovaries enlarged, pale yellow and contain several large Graafian follicles; thymus atrophic; thyroid normal in size and color.

GROUP II: *Protocol III.* Rabbit 217, male; adult

September 17, 1920. Started metabolic studies

October 21, 1920. Under ether removed right suprarenal completely

October 28, 1920. Superficial wound infection

November 16, 1920. Under ether removed left suprarenal completely, enlarged

November 18, 1920. Soft stools, dull, eats fairly well

November 28, 1920. Not eating well, getting weaker, wounds healed

TABLE 1

Rabbit 232

DATE	WEIGHT OF RABBIT.	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS*	CALORIES PER KG. PER HOUR	REMARKS
1920									
December 22.	2916	4.295	5.230	0.52	0.46	88	14.83	2.54	November 26. Right suprarenalectomy (complete)
1921									
January 13..	3153	4.675	4.965	0.52	0.40	77	15.64	2.48	
February 9...	2745	4.085	5.095	0.52	0.47	91	14.07	2.56	
February 21..	2764	4.275	4.855	0.54	0.45	83	14.40	2.60	
February 26..	2712	3.770	4.025	0.49	0.38	78	12.55	2.31	
March 1.....	2812	3.730	4.780	0.46	0.43	93	12.98	2.31	
March 4.....	2843	4.505	5.380	0.55	0.48	87	15.39	2.71	
March 8.....	2839	4.120	4.555	0.51	0.41	80	13.92	2.45	
March 16....	2903	4.515	5.350	0.54	0.47	86	15.44	2.66	
March 24....	2800	4.315	4.920	0.54	0.45	83	14.60	2.61	March 22. Left suprarenalectomy (complete)
March 26....	2780	4.375	5.265	0.55	0.48	88	14.93	2.68	
March 30....	2776	3.970	4.400	0.50	0.40	80	13.44	2.42	
April 4.....	2670	3.645	3.945	0.48	0.38	78	12.30	2.30	
April 15.....	2817	4.040	4.200	0.50	0.38	75	13.51	2.40	

* Calculated from CO_2 for all tables.

TABLE 2

Rabbit 255

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1921									
March 2.....	2998	3.895	4.465	0.45	0.38	83	13.25	2.21	
March 10....	3013	3.555	4.070	0.41	0.34	83	12.08	2.00	
March 21....	3026	3.875	4.225	0.45	0.36	79	13.04	2.15	
March 24....	2917	3.585	3.925	0.43	0.34	79	12.11	2.08	March 22. Right and left suprarenals frozen
March 26....	2953	3.880	4.535	0.46	0.39	85	13.21	2.24	
March 30....	2961	3.745	3.980	0.44	0.34	77	12.54	2.12	
April 4.....	2965	3.660	3.945	0.43	0.34	78	12.30	2.07	
April 15.....	2902	3.695	4.035	0.45	0.35	79	12.45	2.14	

December 27, 1920. Slow deterioration to death on this date, immediate necropsy; thyroids not enlarged; parathyroids normal; patchy consolidation of left lung; right lung free; heart not enlarged; both kidneys are small and "spotted." The spots are due to congested depressions in cortex. On section grayish streaks and focal areas are present both in cortex and pyramidal portion; right and left suprarenals absent; no accessories found; thymus large and cellular; lymph glands of mesentery and retroperitoneum enlarged; no visible abdominal fat.

TABLE 3

Rabbit 217

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1920									
September 17.	2424	3.600	3.877	0.52	0.41	78	12.08	2.49	
November 8..	2590	4.180	4.580	0.57	0.45	79	14.13	2.73	October 21. Right suprarenalectomy complete
November 18.	2450	4.385	4.990	0.63	0.52	83	14.81	3.02	November 16. Left suprarenalectomy complete
November 20.	2482	4.330	4.710	0.61	0.48	79	14.54	2.93	
November 22.	2462	4.410	4.775	0.63	0.49	79	14.74	2.99	
November 24.	2582	4.450	5.010	0.60	0.49	82	15.01	2.91	
December 2..	2478	3.730	4.165	0.53	0.43	81	12.60	2.54	
December 8...	2510	4.000	4.715	0.56	0.48	85	13.73	2.73	
December 15.	2522	4.070	4.965	0.56	0.50	90	13.83	2.74	
December 21..	2466	3.370	3.905	0.48	0.40	85	11.37	2.31	

Protocol IV. Rabbit 237

December 7, 1920. Under ether removed right suprarenal completely

December 9, 1920. Began metabolic studies

January 8–11, 1921. Gave total of 0.4 gram potassium iodid

January 25, 1921. Under ether exposed and froze left suprarenal, with ethyl chloride leaving blood vessels intact

January 30, 1921. Fairly active, eats oats, hay and carrot

February 15, 1921. Active, gave birth to one full-term fetus, destroyed

February 18, 1920. Dull, eats very little

March 15, 1921. In good condition, eats moderately of oats, hay and carrot

March 16, 1921. Died about 6 hours after removal from respiratory chamber, necropsy at once; thyroids small, clear, translucent (iodin effect); parathyroids hyperemic; thymus fatty, little lymphoid tissue; lungs clear; heart not dilated; liver normal in size and consistency, congested; spleen congested; pancreas

hyperemic; left suprarenal present as a flaccid, brownish yellow, encapsulated mass, 10 × 6 × 4 mm., no evidence of any normal cortical tissue; microscopically the tissue is nowhere necrotic; a small oval central area in the lower half of the gland around the suprarenal vein approximately 3.5 × 1.5 mm. is composed of normal-looking medulla, taking a normal chrome stain, extremely vascular. The entire medulla appears to be present; toward the periphery of the medulla mass there are islands of large, granular, normal-staining cortical cells. Beyond this the cortical tissue is shrunken, with numerous cholesterol crystal spaces and cholesterol giant cells; the cortical cells are non-granular, nearly stainless or slightly bluish (with H. and E. stain, the cytoplasm of normal cortex cells takes

TABLE 4

Rabbit 237

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1920									
December 9..	2267	3.625	4.035	0.56	0.45	81	12.20	2.69	December 7. Right suprarenalectomy complete
December 14.	2315	3.685	4.790	0.56	0.53	95	12.79	2.78	
1921									
January 13..	2462	3.460	4.800	0.49	0.50	101	12.33	2.50	Figures on basis of 100 R. Q.
January 27..	2550	3.290	3.595	0.45	0.35	79	11.08	2.17	January 25. Left suprarenal frozen
February 2..	2447	3.635	4.400	0.52	0.46	88	12.47	2.55	
February 8..	2389	3.900	4.540	0.57	0.48	84	13.33	2.79	
February 11..	2455	4.145	5.135	0.61	0.53	90	14.29	2.91	
February 15.	2285	3.795	4.280	0.58	0.48	82	12.82	2.81	
February 18.	2252	3.570	4.485	0.55	0.51	91	12.38	2.75	
February 23.	2212	3.390	4.590	0.54	0.53	98	11.96	2.70	
February 28.	2262	3.320	4.105	0.51	0.46	90	11.43	2.53	
March 8.....	2225	3.355	3.550	0.53	0.41	77	11.18	2.51	
March 16....	2486	3.380	3.635	0.48	0.37	78	11.33	2.28	

eosin); the capillaries are intact and contain blood. This is an excellent example of the preservation of almost if not the entire medulla of the left suprarenal with serious injury of nearly all of the cortex with the preservation of a good blood supply to both parts. Two very small, yellowish white nodules on perirenal fat above and external to kidney; right suprarenal absent; both kidneys show shallow, dark red, pitted areas scattered over cortex; on section there are streaks and rounded areas of greyish lymphoid-like tissue in both the cortical and pyramidal portions; ovaries slightly enlarged, follicles present; abundant fat in all depositories; stomach contains fair amount of food, no ulcers present. Death related to period in metabolism chamber.

Protocol V. Rabbit 206; male; adult

April 22, 1920. Weight 2350 grams. Under ether removed right suprarenal completely

April 29, 1920. Wounds healed—weight 2270 grams

June 7, 1920. Began metabolic studies

October 11, 1920. Under ether removed completely left suprarenal, much enlarged, weight 0.461 gram; animal very mangy, coat dull

October 16, 1920. Eats heartily of oats, hay and carrot, soft stools, mange disappearing

October 20, 1920. Mange nearly gone, coat getting glossy, very active, restless, but fatigues easily, wound healed

October 25, 1920. Eats heartily, drinks more water, periods of fatigue and dullness increasing in duration, although restless and active between, losing weight

TABLE 5

Rabbit 206

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1920									
June 7.....	2390	3.570	3.740	0.52	0.40	76	11.90	2.49	April 22. Right suprarenalectomy complete
June 19.....	2400	3.610	4.170	0.53	0.44	84	12.26	2.55	
September 11.	2743	3.365	3.820	0.43	0.35	83	11.33	2.07	
October 12...	2306	3.245	3.410	0.49	0.38	76	10.85	2.35	October 11. Left suprarenalectomy complete
October 14...	2480	4.365	5.625	0.62	0.60	93	15.27	3.08	
October 19...	2405	4.350	4.837	0.63	0.51	80	14.78	3.07	
October 22...	2275	4.635	4.845	0.71	0.54	76	15.42	3.39	
October 25...	2280	4.344	4.827	0.67	0.54	81	14.60	3.20	

October 28, 1920. Died this morning, immediate necropsy, weight 2197 grams; thyroids not enlarged; parathyroids hyperemic; thymus present and cellular; lungs congested, no consolidation; right heart dilated; spleen normal; liver dark brown red and much reduced in size; pancreas hyperemic; right and left suprarenals absent, wound healing complete; no accessories found; stomach small, contracted, three small ulcers in pyloric portion; intestines nearly empty, contracted; testes small, flaccid; no visible fat in the usual depositories; mesenteric and regional lymph glands prominent; typical suprarenal death.

Protcol VI. Rabbit 251; female; adult

February 15, 1921. Began metabolic studies, coat dull, slight mange and alopecia about eyes

March 14, 1921. Under ether exposed right and left suprarenals, through usual incisions and froze both glands with ethyl chloride; blood vessels left intact.

March 23, 1921. Both wounds completely healed, rapid growth of hair over shaved areas; eats heartily of oats and hay and carrot, is gaining weight rapidly, coat becoming glossy, mange about eyes disappearing

TABLE 6

Rabbit 251

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES PER 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1921									
February 15	2057	3.250	4.215	0.55	0.52	94	11.35	2.76	
February 24	2110	3.420	4.075	0.57	0.49	87	11.67	2.76	
March 4	2157	2.830	4.040	0.46	0.48	104	10.7	2.41	Figured on basis of 100 R. Q.
March 16	2105	3.540	4.365	0.59	0.53	89	12.27	2.91	March 14. Right and left suprarenals frozen
March 18	2283	4.190	6.050	0.64	0.67	105	15.54	3.40	Figured on basis of 100 R. Q.
March 21	2435	4.300	6.180	0.62	0.65	104	15.88	3.26	Figured on basis of 100 R. Q.
March 23	2406	4.430	5.310	0.64	0.56	87	15.19	3.16	
March 25	2556	4.650	6.425	0.64	0.64	100	16.51	3.23	
March 28	2470	4.405	5.585	0.62	0.58	92	15.29	3.10	
March 31	2525	4.145	5.115	0.57	0.52	90	14.25	2.82	
April 2	2599	4.735	6.385	0.63	0.63	98	16.66	3.21	
April 4	2580	4.220	5.090	0.57	0.50	88	14.43	2.80	
April 7	2613	4.150	5.140	0.56	0.50	90	14.31	2.74	
April 11	2627	3.735	4.745	0.50	0.46	92	12.99	2.47	
April 15	2555	3.790	4.070	0.52	0.41	78	12.69	2.51	
April 19	2511	2.975	3.515	0.41	0.36	86	10.14	2.02	
April 21	2507	3.175	3.620	0.44	0.37	82	10.85	2.16	
April 26	2497	3.160	3.570	0.44	0.36	82	10.70	2.14	
May 3	2613	3.585	4.445	0.48	0.43	90	12.38	2.37	
May 9	2491	3.420	3.885	0.48	0.40	82	11.64	2.34	
May 16	2533	3.405	3.990	0.47	0.40	85	11.62	2.29	
May 19	2507	3.150	3.670	0.44	0.37	85	10.69	2.13	

April 15, 1921. Clean, sleek, active, well-nourished, eats entire ration

May 1, 1921. Appetite during last 2 weeks seems increased

May 15, 1921. Seems in perfect health, consumes food ration rapidly

May 20, 1921. Sacrificed, animal apparently in excellent health, necropsy; thyroids and parathyroids of normal size and appearance; thymus present and cellular; lungs and heart normal; spleen soft, possibly slightly enlarged; liver dark brown red, possibly somewhat reduced in size, flabby and tough; kidneys

small and smooth, uniformly grayish brown in color; right suprarenal involved in slight adhesions, measures 10 × 6 × 3 mm.; about half of the gland appears normal while the remainder shows the effect of freezing; left suprarenal easily isolated, measures 11 × 7 × 3 mm., there are large masses of normal looking cortical tissue at each pole, while the middle portion is shrunken yellowish brown from the freezing; no accessories found; ovaries not enlarged; abundant abdominal fat; stomach filled with food, mucosa normal.

GROUP III: *Protocol VII.* Rabbit 257; male; adult

March 14, 1921. Under ether removed right suprarenal completely, enlarged, weight 0.40 grams

March 18, 1921. Began metabolic studies

March 30, 1921. Wound healed completely

TABLE 7

Rabbit 257

DATE	WEIGHT OF RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER kg. PER HOUR	REMARKS
1921									
March 23	2250	3.460	4.715	0.54	0.53	99	12.21	2.71	March 14. Right suprarenalectomy complete
March 31	2290	3.720	4.230	0.57	0.47	83	12.55	2.74	
April 11	2315	3.505	4.125	0.53	0.45	86	11.90	2.57	
April 21	2214	3.660	4.085	0.58	0.47	81	12.36	2.79	
April 30	2105	3.100	3.570	0.51	0.43	84	10.49	2.48	April 28. Left suprarenalectomy complete
May 2	2045	3.160	3.825	0.54	0.48	88	10.84	2.6	
May 5	1965	2.505	2.745	0.45	0.36	80	8.39	2.13	
May 7	1908	2.285	2.470	0.42	0.33	79	7.62	2.00	

April 28, 1921. Under ether removed completely left suprarenal, much enlarged, weight 0.72 gram

April 29, 1921. Active, ate entire ration of oats, hay and carrot

May 1, 1921. Ate very little, looks dull, soft stools

May 7, 1921. Losing weight rapidly, dull, gave 6 grams glucose by mouth

May 8, 1921. Died during night, necropsy; thyroids enlarged and congested; parathyroids congested; thymus present and cellular; lungs and heart normal; liver reduced in size, dark red; spleen normal; pancreas congested; right and left suprarenals completely removed; a 1 mm. grayish, translucent flattened body (accessory cortical mass) on inferior cava just below right renal vein; kidneys appear normal; intestinal tract practically empty; no ulcers in stomach; wounds completely healed; no visible fat anywhere; testes appear normal; typical subacute suprarenal death.

Thyroid Feeding Experiment: *Protocol VIII.* Rabbit 250; adult; female

February 15, 1921. Strong vigorous rabbit. Began metabolic studies

March 4, 1921. Began feeding 0.1 gram very active desiccated sheep thyroid, (containing 0.155 per cent iodin) on alternate days. This thyroid was prepared November 6, 1911, and its pharmacological activity has been tested many times since then, and again this spring on tadpoles, 50 mgm. causing complete metamorphosis in 10 days

March 13, 1921. Looks somewhat dull, fur more erect

March 16, 1921. Gave last of six 0.1 gram doses desiccated thyroid

April 15, 1921. Normal appearance, active

May 15, 1921. Active, healthy, eats all of ration

TABLE 8

Rabbit 250

DATE	WEIGHT AD RABBIT	O_2 IN GRAMS 2 HOURS	CO_2 IN GRAMS 2 HOURS	O_2 PER GRAM PER HOUR IN CC.	CO_2 PER GRAM PER HOUR IN CC.	R. Q.	TOTAL CALORIES 2 HOURS	CALORIES PER KG. PER HOUR	REMARKS
1921									
February 15..	2483	3.940	5.595	0.55	0.57	103	14.37	2.89	Figured on basis of 100 R. Q.
February 23..	2425	3.665	4.195	0.53	0.44	83	12.45	2.57	
March 2.....	2455	3.715	4.475	0.53	0.46	88	12.69	2.58	
March 5.....	2413	4.135	4.595	0.60	0.48	81	13.90	2.86	March 4. 0.1 gram thyroid, alternate days
March 7.....	2419	4.020	4.800	0.58	0.51	87	13.73	2.84	
March 9.....	2477	4.400	5.165	0.62	0.53	85	15.04	3.04	
March 11....	2553	4.200	5.650	0.58	0.56	98	14.74	2.89	
March 13....	2490	4.080	5.250	0.57	0.54	93	14.25	2.86	March 14. Last dose thyroid
March 15....	2586	4.815	6.200	0.65	0.61	94	16.70	3.23	
March 17....	2605	4.030	4.755	0.54	0.46	86	13.72	2.63	
March 19....	2665	4.070	4.850	0.53	0.46	87	13.87	2.60	
March 25....	2575	3.695	4.665	0.50	0.46	94	12.88	2.50	
April 7.....	2731	3.630	4.300	0.46	0.40	86	12.41	2.27	

Group I. This group includes those animals thats urvived indefinitely the operation of double removal or injury of the suprarenal glands and whose respiratory exchanges varied less than 10 per cent from their normal average basal metabolism. Fourteen, or 33 per cent, of the rabbits fall in this group. All of these animals with one exception (rabbit 232) that have died or been killed have shown considerable active cortical tissue, either as accessory suprarenals or as unremoved fragments of the main glands or as cortical tissue which had been unin-

jured in the process of freezing. In view of the previous work of other observers and of our previous experience, it is reasonable to suppose that this animal had accessory cortical tissue somewhere to meet its ordinary demands. The failure to find accessory cortical tissue is of little significance since a complete examination would require microscopic examination of serial sections of the entire area within which cortical tissue might occur.

Protocols I and II (rabbits 232 and 255) have been selected as types. In rabbit 232 both suprarenals were removed at two operations, while in rabbit 255 both suprarenals were frozen at a single operation. In rabbit 232 the right suprarenal was removed 26 days before metabolic studies were begun. A series of nine observations extending over the next 3 months was obtained before, and five observations after removal of the second suprarenal. Both glands were completely removed. The respiratory exchange and the body weight remained relatively constant throughout the 4½ months under observation and death occurred near the end of pregnancy associated with lesions of the kidney and liver closely resembling those of the toxemia of pregnancy. In several instances where pregnancy has supervened in animals surviving double suprarenalectomy in apparent health, death has occurred near the end of pregnancy with the lesions above mentioned.

In rabbit 255, three control observations were obtained over a period of 20 days before freezing and five observations over a period of 24 days after freezing both glands. The respiratory exchange and the body weight remained strikingly constant throughout the period of observation. The animal was apparently in excellent health when killed to terminate the experiment. At necropsy while the greater portion of each suprarenal body was injured, there were masses, approximately 3×3 mm. in the right and 2×2 mm. in the left, of normal suprarenal gland containing both cortex and medulla.

Group II. This group includes those animals that showed, following the double removal or injury of the suprarenal glands, increases in their metabolic rates, followed by a fall to or below the normal whether dying within 2 or 3 weeks or living on indefinitely. This increase in the metabolic rate was observed in 24 animals (of which 7 had one or both glands frozen), or 57 per cent of the series and varied from 10 per cent to 63 per cent above the control. The average maximum increase for the group was 23 per cent.

Protocols III, IV, V, VI (rabbits 206, 217, 237, 251) have been selected as types to show the variations in the degree and duration of the increase

and that these effects may be produced either by removal or injury (freezing) of the suprarenals.

In rabbits 206 and 217 both suprarenals were removed at two operations, while in rabbit 237 the right was removed and the left frozen at two operations and in 251 both were frozen at a single operation. The rise in the metabolic rate usually is manifest on the 2nd or 3rd day while the maximum rise occurred in from 2 days to 3 weeks or more. Sometimes the metabolic rate rose gradually to a peak followed by a gradual fall to or below normal. Not infrequently, however, the rate rose and fell two or three times before finally falling to or below normal.

Rabbit 237 showed a rise of approximately 9 per cent, reaching its maximum on the 17th day after freezing the second suprarenal. Rabbit 217 showed a maximum rise of 12 per cent, reaching its maximum 6 days after removal of the 2nd suprarenal. Rabbit 251 showed a maximum rise of 28 per cent reaching its maximum 4 days after freezing both suprarenals, remained approximately at this level for the next 11 days, then gradually fell to or below normal during the next month. During the period when the heat production was increasing rapidly the respiratory quotients for this rabbit were above 1. The animal was killed to terminate the experiment 67 days after freezing both glands, at which time it appeared normal in all respects.

Rabbit 206 showed a progressive rise reaching its maximum of 42 per cent above its normal on the 11th and 14th days, following removal of the second suprarenal. The animal died on the 17th day without any observations after the 14th day. This rabbit lost weight rapidly although he ate voraciously—an excellent example of rapid tissue as well as food oxidation. The rise in all instances was absolute as indicated by both the total heat production and the CO_2 output.

Certain clinical phenomena have been observed in the animals of this group which are strikingly different from those of other groups. The operation wounds healed more quickly and the hair has grown more rapidly over the shaved areas than those of group 1. The nutrition of the skin improved as shown in certain instances where coats previously dull, dry and mangy became clean and glossy. In many instances the animals showed increased appetite. Soft stools so commonly observed following suprarenalectomy in rabbits were always most pronounced during the period of rising metabolic rate. Animals with increased metabolic rates were more active, more restless, more easily irritated and sometimes became vicious. They likewise became fatigued very

quickly. Evidence of increased sexual activity was also observed. In general the animals showing the greatest rise lost weight in spite of a food intake often larger than their average normal.

Group III includes those animals that survived the operation of double removal or injury of the suprarenals from a few hours to a few days. Only 4 or 9 per cent of the rabbits of the series fall in this group. In the case of cats, dogs and guinea pigs, this group would be much the largest. The metabolic rate shows a fall beginning within 48 hours after suprarenalectomy. In certain experiments a slight rise may precede the fall, in others this is absent. The rapidly fatal outcome and the fall in the respiratory exchange are parallel phenomena and depend, it is believed, upon the completeness of destruction of cortical function. Protocol VII (rabbit 257) showing a fall of 26 per cent in heat production below its normal illustrates the group. The period of survival was somewhat prolonged (9 days) but on this account the progressive fall in the metabolic rate is more definite.

Thyroid feeding. Protocol VIII (rabbit 250) is introduced to show the effect on the respiratory exchange of feeding desiccated thyroid to normal rabbits and for comparsion with the effects of suprarenalectomy. One-tenth gram of an active preparation was given by mouth every other day for 12 days. An irregular rise in the heat production reaching 20 per cent above the control rate on the 11th day resulted. The desiccated thyroid was discontinued after 6 doses and the rate rapidly returned to the normal average. The rise in the heat production following the thyroid feeding was less than the average obtained following suprarenalectomy.

DISCUSSION

It appears established that by removing or crippling (by freezing) the suprarenal mechanism a disturbance in the metabolic rate characterized by an increased heat production may be brought about. This disturbance appears definitely related to the completeness of removal of suprarenal function and the duration of life. If only one gland is removed usually no noteworthy changes follow. Even when both are removed there may be no change in the animal's behavior or in its metabolic rate. In such cases one can almost always demonstrate the presence of accessory masses of suprarenal cortex. In most instances (57 per cent of this series) where both glands were removed or crippled there was a progressive rise in the metabolic rate. Sixty-eight per cent of the rabbits in which both suprarenals were removed and 41

per cent of those in which one or both suprarenals were frozen showed an increased heat production. This rise is highly variable both in degree and in duration—no two rabbits showing the same response, irrespective of age or sex. Rabbits show wide variations in their normal basal metabolism though it is relatively constant for a given rabbit over a long period of time. In our series "normals" have varied between 2.1 and 3.2 calories per kilogram per hour, the mean and average being 2.5 calories. Eighty-five per cent varied within 12 per cent of the average.

The increased metabolic rate following suprarenal injury may continue for 1, 2, 3 or more weeks with or without slight remissions and is succeeded by a gradual fall continuing to death or if the animal survives indefinitely, to or slightly below its control rate.[2] Occasionally rabbits surviving indefinitely show more than one period of increased metabolism before the metabolic rates finally become stable at or below the control level. In animals dying within a week following suprarenal injury a rise may not be detected (if observations are made on alternate days, as was the usual procedure in these experiments) or if present, may be quickly succeeded by a decrease continuing until death.

Infection or febrile reaction of other origin or trauma of operation may be eliminated as a cause for the rise. The trauma of operation was approximately the same throughout this series, whether removal was complete or not. But in those where complete removal of the right suprarenal was impossible because of its location, no change in metabolic rate was observed following removal of the second.

The protocol showing the effect of feeding desiccated thyroid was introduced in order to compare the effects on the metabolic rate produced by thyroid feeding with those produced by suprarenal injury. The rise resulting from thyroid feeding was 20 per cent in this instance, which is much less than has been frequently obtained following suprarenalectomy, although the dose used was large and pharmacologically the preparation was very active as shown by its ability to cause complete metamorphosis of tadpoles within 10 days. The comparatively small rise in heat production caused by thyroid feeding suggests that the rabbit is not highly susceptible or is incapable of reacting to the same extent that other animals do. If either of these suggestions is true, and

[2] Rabbits, though to a lesser extent than cats and dogs, show the effect of training. They are more restless the first two or three times they are placed in the chamber. The higher control rates and the lower metabolic rates after a few weeks or months may be thus partially explained.

if the increased heat production following suprarenalectomy depends upon the thyroid, it is probable that animals more susceptible to thyroid feeding will show greater reactions following injury to the suprarenals than will rabbits.

No conclusions have been arrived at regarding the mechanism of the rise in the metabolic rate following suprarenal injury. We have developed a working hypothesis which is as follows: Supposing the intact thyroid gland to be the major factor in the maintenance of a given metabolic rate and that this function is controlled by some regulatory mechanism, we have argued that possibly this control might be exercised by a restraining influence, a major factor in which was the normal function of the suprarenal glands. If such should be the case, the removal or crippling of this restraining or inhibitory influence would allow the thyroid to increase its activity. The frequency with which thyroid and thymus hypertrophy occur after suprarenalectomy in rabbits is additional evidence of the possibility of increased thyroid activity. One cannot neglect to consider the possibility, indeed the probability, of vastly more extensive disturbances in the functions and interrelationships of other organs than the thyroid. There is other evidence that suggests a more general regulatory or inhibitory function of the suprarenal gland. In suprarenalectomized animals there is increased sexual desire, increased intestinal peristalsis associated with soft stools, and increased mobilization of sugar. Wound healing and the growth of hair over shaved areas may be more rapid than ordinarily. The fur over the whole body often became smooth and more glossy. Such animals are usually more irritable and even become vicious. There is sometimes evidence of increased appetite. We have noticed instances where in association with a rise of the metabolic rate there was an increase in body weight associated with respiratory quotients above 1. All these phenomena might be interpreted as suggesting that the suprarenal glands may normally exercise a regulatory action over many body functions. Renal lesions have been observed in a significant number of rabbits surviving suprarenalectomy from 3 to 5 weeks. They have not been detected in rabbits dying within 10 days. The changes have been briefly described in protocols III and IV. Marshall and Davis (10), working with cats, showed there was a definite increase in the urea and creatinin content of the blood in suprarenalectomized cats, but were unable to detect any renal lesions, though carefully looked for. In rabbits we have also observed distinct increases in the urea and creatinin content of the blood in animals after

the onset of the marked terminal asthenia, although at the height of the increased metabolism these substances usually are within normal limits. The possibility of injury to the renal function and subsequent retention of products of metabolism being factors in the increased heat production has not been eliminated inasmuch as Peabody, Meyer and DuBois (11) have shown that occasionally there is an increase in the basal metabolism in cardio-renal cases with cardiac decompensation.

Another question of primary importance arises, namely, is it the medullary or cortical influence which is primarily concerned in the above mentioned disturbance of metabolism. All the evidence at present available indicates that the cortex is the important and the medulla the unimportant tissue as regards maintenance of life. Only those animals survive suprarenalectomy who have functionally active cortex. Epinephrin does not prolong life after suprarenalectomy, and further, the discharge of epinephrin may be nearly if not completely abolished without apparent injury to the animal. As has already been pointed out, if a portion of the cortex, or if only accessories are present, the rise of the metabolic rate may not occur. Also, if most of the cortex is crippled by freezing, leaving the greater part of the medulla intact, as determined histologically, a marked rise may occur. This evidence, although meager, points to the cortex both as the tissue necessary to life and to its being more intimately related to the increased heat production than the medulla. However, freezing experiments do not exclude the medulla as an important factor in this phenomenon since the nerves going to the glands are also frozen and many investigators have shown that the discharge of epinephrin is dependent upon intact nerves (12).

Finally there are many points of similarity between the symptom complex that results from suprarenal injury as above described in rabbits and exophthalmic goiter in man. This possible relationship will be presented in a separate paper.

CONCLUSIONS

1. Removing or crippling (by freezing) the suprarenal glands in rabbits causes a disturbance in metabolism, usually characterized by increased heat production and CO_2 output.

2. This disturbance appears definitely related to the completeness of removal of the cortical function.

3. The symptom complex including both anatomical and physiological data which results from the destruction of the suprarenal function in rabbits resembles in many essential features the symptom complex of exophthalmic goiter.

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STUDIES ON NEUROMUSCULAR TRANSMISSION

I. The Action of Novocaine on Muscle Nuclei[1]

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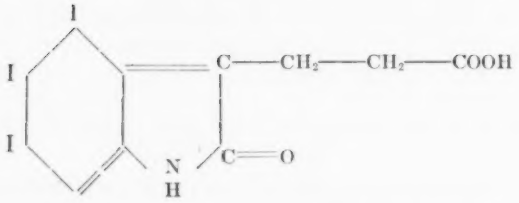
Introduction. One of the most interesting phases in the growth of biological chemistry is the study of the relation of physiological activity to chemical constitution; that is, the relation between the structure of the molecule and the effect which it produces on the organism. The pioneer in this field of research was Paul Ehrlich, and to his industry and resourcefulness the medical profession owes a debt which it has never fully recognized. His contributions to the study of anemia, immunity and syphilis are well known to professional men, but his theory of physiological activity has never received the attention which it seems to me to deserve, probably because it has not been adequately confirmed by experimental proof.

A drug,[2] said Ehrlich, to be physiologically active, must possess within its molecule two chemical groups; one, a physiologically active group and the other an "anchoring" group. It is the function of the anchoring group to fix the drug to the tissue in which it is to act, and it must, therefore, have a particular affinity for that tissue. The physiologically active group, on the other hand, is, as the name indicates, the portion of the molecule which is responsible for the action of the drug. An interesting example of a substance whose physiological activity may be interpreted in the light of this theory is thyroxin, the active principle of the thyroid gland. The recent investigations of Kendall (19) have shown the structural formula of this substance to be:

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, No. 332, and from the Bermuda Biological Station for Research, No. 128.

[2] The best elucidation of Ehrlich's theory is to be found in his *Studies in Immunity* (8). An estimate of Ehrlich's work has recently appeared in the *Proceedings of the Royal Society*, London, January, 1921.



From what is known of the importance of the iodin atom in the animal body, e.g., in the metamorphosis of tadpoles,[3] and in certain diseases of the thyroid,—it is reasonable to look upon the group which contains the iodin atom as the physiologically active constituent of the molecule; while the remainder of the molecule may be regarded as the anchoring constituent. Moreover, when the anchoring group of thyroxin is altered, the substance loses completely its physiological activity. Thus, in alkaline solution, the imino ring (NH) opens and the substance becomes physiologically inert even though the iodin atoms are still present. This is to be explained by assuming that the alteration of the anchoring group causes it to lose its affinity for the tissues to which it usually attaches itself.

Inasmuch as satisfactory experimental proof for Ehrlich's theory has hitherto been wanting, it appeared desirable to record any experimental evidence which might bear upon the theory.

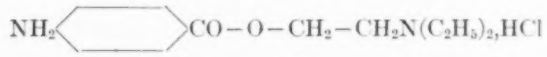
It occurred to the writer that if certain plant alkaloids or anesthetics which are generally supposed to have a special affinity for nervous tissue, could be linked chemically with a staining base, a compound would result which would be a specific nerve stain. In the language of Ehrlich, the alkaloidal constituent of the molecule would be the anchoring group, and the staining constituent, the physiologically active group. Accordingly, the chemistry of the alkaloids and anesthetics was thoroughly inspected in an effort to find one which would be readily amenable to chemical linkage.[4] For this purpose, the most desirable way of linking an organic compound with a staining base is by means of a process known as diazotization. To be diazotized, a substance must possess a benzene nucleus which has attached to it a free amine

[3] A review of the work on the relation of iodin to the metamorphosis of tadpoles will be found in a paper by the writer (10).

[4] The most useful works on the chemistry of alkaloids and anesthetics which have come to my attention are Henry's *Plant Alkaloids* (15) and May's *Chemistry of the Synthetic Drugs* (26).

(NH_2) group. This, when acted upon by nitrous acid, forms the characteristic diazo ($N \equiv N$) group. When placed in the presence of another benzene ring, this diazo constituent becomes linked with it and forms a colored compound. Consequently, if it were possible to find a substance specific for nervous tissue which has a free amine group attached to a benzene nucleus, it could be linked very simply with a staining base.

Diazotization. Of the substances which act upon nervous tissue in the capacity of anesthetics, novocaine appeared to be the most promising for this investigation, since it possesses the necessary prerequisites for diazotization: a free amine group attached to a benzene nucleus. The following formula shows the structure of novocaine:



Having selected novocaine as the anchoring group for the nerve stain, it remained to discover a suitable staining base to link with it. Inasmuch as there are literally thousands of compounds from which to choose, it was almost necessary to resort to a method of trial and error in making the selection. The resulting compound of the staining base and the novocaine must in the first place be soluble in water; it must also retain the physiological activity of novocaine. The diazotization was first performed in succession with *beta*-naphthol, phenol and resorcine, but, owing to the slight solubility of the stains which were produced, they had to be rejected. Neville and Winther's acid was also tried, but with negative results; *alpha*-naphthylamine and *beta*-naphthylamine were little more satisfactory. The next staining base which was tried was a diamine, selected because, as a general rule, diamines are highly soluble in water (urea) and also readily penetrate animal tissues. Such a compound is *meta*-phenylenediamine, and it was found that when linked with novocaine by diazotization, a brown crystalline substance is produced which is highly soluble in water. Table 1 gives a list of the various combinations of anesthetics and staining bases made in the course of the present work. It will be seen that not only novocaine, but also subcutin and propaesin were employed as anchoring constituents in the molecule. The compounds which resulted from the diazotization of the last two anesthetics were in every case only sparingly soluble in water. It remained, therefore, to investigate more extensively the *meta*-phenylenediamine compound of novocaine.

TABLE 1

The diazotization products of novacaine, subcutin and propaesin with various staining bases

ANESTHETIC	STAINING BASE	PROPERTIES	
		Color	Solubility in water
Novocaine	*beta*-Naphthol	Crimson	Insoluble
Novocaine	Phenol	Yellow	Insoluble
Novocaine	Resorcine	Brown	Soluble (1 per cent)
Novocaine	Neville and Winther's acid	Brown	Insoluble
Novocaine	*alpha*-Naphthylamine	Reddish purple	Slightly soluble
Novocaine	*beta*-Naphthylamine	Reddish purple	Slightly soluble
Novocaine	*meta*-Phenylenediamine	Brown	Readily soluble
Subcutin*	*alpha*-Naphthylamine	Purple	Insoluble
Subcutin	*beta*-Naphthylamine	Deep red	Insoluble
Subcutin	*meta*-Phenylenediamine	Bichromate	Insoluble
Propaesin†	*alpha*-Naphthylamine	Pale brown	Insoluble
Propaesin	*beta*-Naphthylamine	Brick red	Insoluble
Propaesin	*meta*-Phenylenediamine	Purple brown	Insoluble

* The formula for subcutin is: $NH_2.C_6H_4.COOC_2H_5$, $HO.C_6H_4.SO_3H$. A description of its synthesis is to be found in May (26, p. 102).

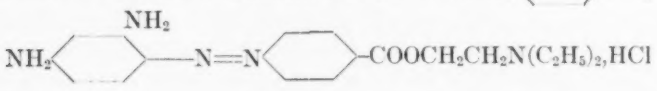
† Propaesin is the propyl ester of *para*-amino-benzoic acid and is represented by the formula: $NH_2C_6H_4COOC_3H_7$.

The chemical reaction involved in the diazotization of novocaine with *meta*-phenylenediamine may be shown as follows:

(1) $NH_2C_6H_4COOCH_2CH_2N(C_2H_5)_2,HCl + HONO \longrightarrow$

$$N{\equiv}N{-}C_6H_5COOCH_2CH_2N(C_2H_5)_2,HCl + H_2O$$

(2) $N{\equiv}N - C_6H_5\ COOCH_2CH_2N(C_2H_5)_2,HCl + NH_2C_6H_4 \longrightarrow$



"Novocaine Brown"

The point on the ring at which *meta*-phenylenediamine will link is, as shown in the last formula, *para* to the first amine group and *meta* to the second. This was concluded from the fact, well-known to the organic chemist, that in diazotization *meta*-phenylenediamine always links in this position (4, pp. 21 and 356).

PHYSIOLOGICAL ACTION OF THE DIAZOTIZED NOVOCAINE. Having found that the *meta*-phenylenediamine salt of novocaine (which hereafter will be referred to as "novocaine brown") is freely soluble in water, the problem of its physiological activity immediately presented itself. Are the physiological properties of novocaine brown the same as those of novocaine? A series of experiments was undertaken to test the physiological activity of this dye, and the general conclusion was reached that novocaine brown possesses all the physiological properties of novocaine. The evidence for this came from several sources.

1. It is well known that novocaine, when injected into the body cavity of the Killifish, *Fundulus*, causes the pigment cells (chromatophores) of the scales to expand immediately, giving to the fish its characteristic black coloration. Likewise in the isolated scale novocaine causes the pigment cells to expand. Novocaine brown also produces exactly this expansion, and its reaction-time is practically the same as that of novocaine.

2. When applied directly to the gastrocnemius muscle of the frog, novocaine destroys the reactivity of the muscle to nerve stimuli. To demonstrate this, one makes a preparation of the gastrocnemius muscle with the sciatic nerve attached to it. When the nerve is stimulated with an electric current, the muscle contracts. If, however, the muscle is first painted with a 5 per cent aqueous solution of novocaine, after 10 minutes the muscle will no longer respond when the nerve is stimulated. If novocaine brown is applied in the same way, it produces a like result.

3. It has recently been shown by Göthlin (12) that the plates of cilia in the Ctenophore Beroë when stimulated by a suitable electric current immediately stop beating. However, if the tissue is first treated with atropine or chloral hydrate, the electrical stimulation does not inhibit the ciliary beat; in fact, instances were observed in which there was an actual acceleration of beat in response to the faradic current. Novocaine appears to produce the same effect as the substances which Göthlin employed for, as I have demonstrated, after it has been applied to the mantle of a clam, electrical stimulation of the mantle causes no visible changes in the beating of the cilia. In this regard novocaine brown is equally effective.

From this evidence it seems a reasonable conclusion that novocaine brown retains the properties of novocaine. If, therefore, the assumption made in the beginning of this paper be true,—viz., that novocaine

has an especial affinity for nervous tissue,—novocaine brown should itself possess the properties of a specific nerve stain.

A HISTOLOGICAL STAIN. *1. The prepared stain:* A large number of tissues of frogs and tadpoles in the living state were treated with the prepared stain, and it appeared to have no affinity for nervous tissue. In fact, only the nuclei of the tissues took the stain. Smears made from the spinal cord, sections of the retina and cornea of the eye were treated, and only the nuclei were stained, never the nerve fibers. The possibility therefore suggested itself that novocaine enters all tissues with equal facility but acts only on nervous tissues. In an effort to test this possibility, a process of intra-vital diazotization was resorted to.

2. Intra-vital diazotization: By putting living tissue first into novocaine then into nitrous acid, (washing thoroughly after each reagent), and finally into the staining base, it was found that the stain could be diazotized very successfully directly into the living tissue. By such a process extremely delicate differentiation is possible and in addition the stain is obtained within the tissue in extreme purity. By varying the length of time in which the tissue is allowed to remain in novocaine, and varying also the period of washing, any desired depth of stain may be procured. The first wash removes all of the excess novocaine. If washed a sufficient length of time, moreover, it may be assumed that only that portion of the novocaine which is fixed firmly in the tissue for which it has a particular affinity will remain. In every case it was found that the novocaine had concentrated itself in the nuclei of the cells and never in the nerve fibers. The result of this method is exactly the same as that obtained when the prepared dye is used, save that the elements are more clearly stained.

If cross sections of tadpole material (mounted upon slides) are diazotized directly with novocaine and *meta*-phenylenediamine, an extremely clear histological picture of the material results and, as in the living tissue, the degree of differentiation may be very satisfactorily controlled by altering the time during which the tissue is permitted to remain in novocaine and also by varying the period of washing. The most satisfactory results for such material are to be obtained by leaving the slide in the various solutions for the following periods:

Novocaine (3 per cent)	5 minutes
Wash in water	2 minutes
Nitrous acid	1 minute
Wash in water	2 to 3 seconds
meta-Phenylenediamine[5] (cold)	1 to 30 minutes

[5] One per cent solution in distilled water.

The nitrous acid is formed by adding 2 or 3 drops of weak hydrochloric acid to a 1 per cent solution of sodium nitrite in a Coplin jar. The acid is not added until immediately before using.

From the staining experiments it was at once evident that novocaine does not act upon the nerve fiber. It appeared desirable, therefore, to determine the activity of novocaine physiologically. If novocaine acts only on the nuclei of tissue, it follows that in a tissue devoid of nuclei it should have no physiological effect. Consequently, if a nerve trunk, such as the sciatic, which is composed of nerve fibers without nuclear material, is soaked in novocaine, the power to conduct impulses should not be abolished by the anesthetic.

THE ACTION OF NOVOCAINE UPON NERVE AND MUSCLE. The validity of this assumption regarding the physiological action of novocaine was tested by treating the several constituents of the neuro-muscular mechanism of a frog with novocaine. Sciatic-gastrocnemius preparations were made; the sciatic nerve was then immersed in 5 per cent solution of novocaine, with the result that no visible diminution in its conductivity was to be observed in the course of from half to three-quarters of an hour. This made it evident that novocaine does not inhibit the passage of motor nerve impulses. It had been noted previously that if the gastrocnemius muscle were immersed in novocaine, it could no longer respond to nerve stimulation. However, if the muscle is stimulated directly (by touching the electrodes to the muscle) it contracts readily. This indicates that novocaine, though it prevents the reception by the muscle of nerve stimuli, does not abolish the power of contraction. Inasmuch as the anesthetic does not impede the passage of the nerve impulse, one must conclude that novocaine paralyzes neither the nerve-fiber nor the contractile element of the muscle fiber, but some substance between the two. Since the motor nerves terminate at the periphery of the muscle fibers in structures known as end plates, it would seem that the membrane which separates the end-plate from muscle fiber might be the seat of activity of the novocaine.

It is a curious fact that the Indian-arrow poison, curare, as originally shown by Claude Bernard (1),[6] also acts upon some structure inter-

[6] Pelouze et Bernard (27) were the first to point out that stimulation of the nerves had no effect on a frog which previously had been injected with curare. The explanation of the activity of curare was not published until 1857. Meanwhile, Kölliker (18) had published the details of his experiments with curare. The results of the two investigators were similar. The more recent work on the physiological activity of curare is to be found in the following papers: Langley (22), (23); Garten (11); Cathcart and Clark (5).

mediate between nerve and muscle. Curare, like novocaine, does not affect nerve transmission nor does it destroy the power of muscular response to direct stimulation. Inasmuch as the late war has occasioned a keenly-felt shortage of curare, it seems desirable to emphasize the fact that novocaine offers a very satisfactory substitute for a substance which at the present time is practically unobtainable.

The activity of curare has been explained on the hypothesis that intermediate between a nerve and its muscle there exists a substance, or possibly an actual membrane, which concerns itself with the reception and transmission of nerve stimuli to the muscle proper. The presence of such a substance was long ago urged, and its existence was demonstrated with reasonable satisfaction by Langley (21). In his earlier researches Langley held that an actual membrane existed between the nerve and the muscle, but later he abandoned this idea, and adopted the view that there exists a "receptive substance," which is possibly akin to a chemical side chain,—the side chain, perhaps, of the molecule of which the contractile substance of the muscle fiber itself is composed. The investigations of Lucas and Adrian (25) leave no doubt as to the actual existence of such a receptive substance. They have been able to show by very delicate experiments that the rate of transmission of impulses within this receptive substance is markedly different from the transmission of impulses either in the nerve itself or in the muscle fiber. However, the exact location of the receptive substance has never been definitely ascertained. As a result of the original conception of a membrane separating the nerve fiber from the muscle, it has usually been taken for granted that the place of reception of nerve stimuli is at the surface of the muscle fiber. Indeed, morphologically the nerve fiber certainly ends at the periphery of the muscle. As far as the writer is aware, it has not hitherto been suspected that the receptive substance resides within the muscle fiber itself. Elliott (9, p. 467), however, has noted in his experiments with adrenalin (which he found to act upon the junction between nerve and muscle) that "the irritable substance at the myoneural junction depends for the continuance of its life on the nucleoplasm of the muscle cell, not of the nerve cell." Aside from this observation there appear to be no other experimental results which point to the nucleus as the seat of the receptive substance. It is, however, of interest that Edmunds and Roth (7, p. 45), as a result of their experiments on nicotine-curare antagonism in the muscles of fowls, find that curare acts "upon the muscle substance proper."

It would be decidedly unwise, in a question which is of such great importance, to draw any but well substantiated conclusions, particularly inasmuch as the experiments herein recorded suggest a relationship between the nerve and muscle which differs fundamentally from that which is accepted by most physiologists. The beliefs which are about to be expressed, therefore, are not intended in any way as final, but rather as a working hypothesis, which further experimentation will probably modify.

As has been already noted, morphologically the nerve ends at the surface of the muscle fiber, and, as far as is known, there is no specialized structural continuity between the peripheral membrane and the nuclei within the muscle. Yet novocaine, which destroys the power of the muscle to receive nervous stimuli, acts upon muscle nuclei and apparently acts only there. The evidence for this comes most strongly from the experiment in which a living muscle soaked with novocaine is directly diazotized with a staining base. From the color distribution of the resulting compound, the seat of activity seems very definitely to be the muscle nuclei. Moreover, when the process of diazotization is reversed—that is, by placing the muscle first into the staining base and then into the diazotized novocaine—a diffuse color results, which is not concentrated in any of the tissue elements. It seems certain, *therefore, that novocaine prevents the reception by the muscle of nerve stimuli, because of its action upon the nuclei of the muscle fibers.* With this observation in mind, what can be said of the means of nervous excitation of striated muscle?

It seems probable, in the first place, that the much discussed "receptive substance" of Langley is located within the nuclei of the muscle and, after being acted upon by novocaine, no longer retains the power of exciting the muscle when the nerve is stimulated. Thus, though there may be doubt concerning the existence of a specialized morphological connection between the nerve ending and the nuclear substance of the muscle fiber, it appears that physiologically they are connected most intimately. Moreover, if this view is substantiated, it will demonstrate that the immediate mechanism by which normal muscular contraction is occasioned is the muscle nucleus itself, and that nerve stimuli are received by the muscle nuclei because of the receptive substance residing within them.

The histological conditions which have been demonstrated from time to time tend strongly to support the view that the nerve communicates its impulses to a muscle through the medium of the muscle

nucleus. The axis-cylinder of the motor nerve, it is now agreed, terminates beneath the sarcolemma in an expanded end. Associated with this expanded end are always to be found the "end-plate," or "sole" nuclei (17). In the light of the work on novocaine it is singularly suggestive that there should be such close morphological relation between the motor terminations and the end-plate nuclei. Of much greater significance, however, is the fact that the end-plate nuclei appear to be identical histologically with the nuclei of the muscle fiber proper. Indeed, Huber and DeWitt (17, p. 179) have reached the "conclusion that what has been described as granular sole [end-plate sarcoplasm] may be regarded as an accumulation of sarcoplasma of the muscle. The nuclei of the sole are in structure very similar to the *muscle nuclei, and we have regarded them as such.*" Concerning the motor endings in the striated muscle of the frog Huber and DeWitt (17, p. 183) have again made a most important observation: "In sections giving a surface view of the muscle fibers of the frog, double stained in methylene and alum carmine, it may be seen that the muscle nuclei are more numerous in that portion of the muscle fiber receiving the ramified endings of the motor nerve. Many of these nuclei are found in the thin layer of sarcoplasma in which the axis-cylinder branches terminate. These nuclei are comparable to the so-called sole nuclei, which, it will be remembered, were also regarded as nuclei of the sacroplasma—muscle nuclei."

Other evidence which parallels this is found in the innervation of the heart. "Within the sinus node [the sino-auricular node] . . . . nerve fibers enter . . . . and divide into delicate plexuses from which varicose fibers terminate in simple or complex fashion around the nuclei of the muscle cells" (31, pp. 27–28). Also some of the fibrils of the His-Tawara system "end around the nuclei of the muscle cells."

THE EFFECT OF NOVOCAINE ON THE HEART OF THE CHICK BEFORE AND AFTER INNERVATION. One of the most hotly debated controversies which ever occurred in connection with physiological investigations arose over the question of the origin of the heart-beat. Though of little concern at the present time, the question is still of considerable historical interest. The most important evidence in favor of the myogenic theory of the heart beat came from the observation of His (16), that the heart of the embryonic chick commences beating after 2 days of development, while it does not become innervated until the 6th day. Thus, in the chick there is a very clear instance of heart muscle which beats before nerves reach it.

If, now, novocaine serves only to block the reception by the muscle of the nerve impulse, it should have no effect upon the beating chick heart before it becomes innervated. Embryos of chicks, 3 days old, were very carefully removed from the shell and placed in physiological salt solution, where they were maintained at the incubation temperature; the activity of the heart was observed through a microscope which was so enclosed in a chamber as to keep the desired temperature of 37°C. The rate at which the heart was beating (usually about 50 times a minute) was recorded and then a warm solution of 5 per cent novocaine added directly to the salt solution in the vicinity of the beating heart. There followed, after a few moments, a spasmodic series of contractions, due probably to the alteration in the osmotic pressure occasioned by the novocaine. Within 5 minutes, however, the normal beat became reëstablished. Subsequent additions of novocaine did not seem to affect the beat, and the heart continued its activity in this condition for more than a half-hour.

As was predicted, therefore, novocaine does not affect the contraction rate of the uninnervated chick heart, a fact which gives support to the contention that novocaine destroys only the power to receive nerve stimuli.

On the innervated chick heart, from 6 to 10 days of age, novocaine appears also to be without effect, for when applied directly to a beating heart, which had been removed altogether from the chick embryo, there followed no alteration in the rate of beat. It appears likely, therefore, that novocaine does not affect the heart beat either of embryonic or mature animals; it might well be suspected, however, that after treatment with novocaine, the nervous control of the heart is suspended. To investigate this, the beating heart of a frog was bathed in a strong solution of novocaine (there followed no marked change in the rate of beat)[7] and the vagus nerve exposed for purposes of stimulation. The vagus nerve was first stimulated to test its influence on the heart and having demonstrated satisfactorily that the usual inhibitory effect followed stimulation, the heart was bathed with strong novocaine. After 5 or 6 minutes, the stimulation of the vagus produced no effect upon the pulsation of the heart. Novocaine, therefore, removes the

[7] Roth (29) has made an extensive study of the effect of novocaine on the heart beat of the frog, and finds that when perfused with a strong solution of novocaine, the rate of pulsation is decreased slightly, and that the ventricular contraction is somewhat reduced. When large doses are injected intravenously in higher animals, there follows a depression in the pulse (14).

power of inhibition on the part of the vagus nerve. As in the case of transmission in the sciatic nerve, novocaine does not interfere with the conduction of a nerve impulse in the vagus; consequently, the seat of activity of the novocaine must here also reside in the receptive substance intermediate between the free endings of the vagus and the cardiac tissue. It is of interest that atropine likewise abolishes the inhibitory effect of stimulation of the vagus trunk (21), and that it also is believed to act upon the receptive substance. In the case of cardiac tissue it can very easily be shown by intra-vital diazotization that novocaine has the same action on the muscle nuclei of the heart as in the case of the gastrocnemius, and therefore that the seat of its activity is the same in both cases. It seems reasonable, therefore, to believe that atropine, which acts on the receptive substance, must also act through the nuclei.

The experiments of Göthlin (12) cited above are of especial interest in this connection. When a ciliated tissue is stimulated electrically after treatment with atropine (or novocaine) the cilia continue to beat as usual; when stimulated without such previous treatment, however, their beating stops at once. It has long been known that in certain ciliated tissues there are definite morphological structures connecting the basal bodies of cilia with the nuclei of the cells. Since, therefore, novocaine and atropine remove ciliary inhibition, it is probable that they do so by acting upon the cilia through the nucleus; that the faradic stimulus affects first the nucleus, which in turn inhibits the ciliary beat. If such be the case, it is clear that in ciliated tissue there is a mechanism which apparently duplicates in a surprising way the contractile mechanism found in muscular tissue, where the nucleus appears to excite the cytoplasmic elements of the cell to functional activity.

THE EFFECT OF NOVOCAINE ON SENSORY TERMINATIONS. Medicinally, novocaine is used as a local anesthetic,[8] and from a practical standpoint, the professional man is more concerned with its effect upon sensory nerve endings than upon motor endings. The experimental results recorded in the present paper deal almost entirely with the effect of novocaine upon motor nerves. In the course of the investigation, however, it seemed advisable to determine whether or not novocaine had a greater affinity for sensory nerve fibers than for motor. About one-quarter of the fibers within the sciatic trunk are sensory. Consequently, when the sciatic nerve is stimulated on one side, the

[8] A discussion of the medicinal uses of novocaine is to be found in Gwathmey's book on anesthesia (13).

leg on the opposite side responds as a result of reflex action. This is due to the well-known fact that when the sensory fibers of the sciatic nerve are stimulated, they transmit the stimulus to the spinal cord, and that, passing through the reflex centers, the stimulus travels down the motor nerve on the opposite side of the body and causes the contraction of the leg muscles. If, before stimulation, the sciatic nerve is treated with novocaine, there is some evidence that the intensity of the reflex is slightly diminished, which possibly indicates that novocaine does interfere with the conduction of such sensory stimuli as are produced by an electric shock.

Though of uncertain effect upon sensory fibers, there is little doubt concerning the action of novocaine upon sensory terminations. When the gastrocnemius muscle on one side of the body is stimulated directly, a prompt reflex is produced on the opposite side; this means that the sensory terminations within the gastrocnemius have received the impulse and have transmitted it through their fibers to the other leg. The same reflex response is secured if the sciatic trunk is exposed above the gastrocnemius and stimulated. If the gastrocnemius is now bathed in novocaine no reflex is produced by direct stimulation of the muscle. The exposed portion of the sciatic trunk, however, will still transmit impulses to the opposite side when stimulated. It is clear, therefore, that in the frog novocaine acts upon the sensory terminations, but has little, if any, effect upon the actual transmission of sensory impulses. It appears to act upon the terminations in such a way as to prevent the reception of impulses, and it is this fact which undoubtedly accounts for its activity in local anesthesia.[9]

In an extensive investigation on the physiological effect of novocaine, Liljestrand und Magnus (24) find that, when injected into the blood stream of a cat, it appears to act upon the proprioceptive (sensory)

[9] It is well known that when a nerve trunk in man is perfused with novocaine, there ensues a complete loss of sensation in all of the tissues which the nerve supplies; however, it does not inhibit materially the passage of such 'sensory' impulses as are produced by inductorium. This result is perhaps the most difficult of all to reconcile with the facts already known concerning the physiological action of novocaine, and it suggests that a normal sensory impulse differs fundamentally in its nature from a motor impulse, or at least from such an impulse as is incited by a faradic current, the true sensory impulses being blocked by novocaine, while motor impulses and those—both sensory and motor—occasioned by an electric shock are quite unaffected by the presence of the anesthetic. Further investigations are now being made in an effort to explain these discordant results.

roots, and in that way destroys the muscle sense. They note also that in large doses it seems to paralyze motor-nerve transmission. The latter result has also been observed by Kubota and Macht (20). It is also interesting to recall that Dixon (6) found that cocaine acted upon sensory nerves more readily than upon motor.

It may very well be, however, that the reception of sensory stimuli by the afferent nerve endings is, as in the case of the efferent endings, effected through the nuclei of the receptive tissues. This would mean that the abolition of sensation by novocaine is due to its action on the nuclei rather than on the sensory nerve endings. The writer, however, does not wish to emphasize this possibility until more substantial evidence has been procured. The fact remains, however, that novocaine when diazotized appears to have no affinity whatsoever for sensory or motor nerve endings.

DISCUSSION

If the observations on the diazotization of novocaine were the only evidence upon which to base the generalization that the receptive substance of Langley is located within the muscle nuclei, the writer would be extremely reluctant to put it forward even as an hypothesis. There are, however, other arguments in favor of the theory which by many will be considered much more convincing than the staining results. This is particularly true of the morphological evidence. The fact that muscle nuclei are crowded into the end-plate is a most suggestive condition and, as far as the writer is aware, no one has hitherto attempted to account for it. In the light of the present theory this concentration of nuclei is readily explained by assuming that they have migrated from the muscle fiber to come more closely into association with the motor terminations. It is extremely interesting, too, that the end-plate as such is found only in muscles of great activity. They are never encountered in smooth muscle of any animal; and in sluggish animals are seldom found even in the voluntary muscles. In the frog the end-plates are best formed in its most active muscles, particularly in the gastrocnemius. Even here, however, the end-plates are rudimentary as compared with those of the higher mammals (see Huber 17. pl. 14; ånd Boeke, (2, Taf. 17–23); and (3)). An examination of the figures of those who have worked upon the motor terminations makes it evident that the end-plate has increased in complexity, particularly as regards the number of nuclei which it contains, as the muscles have increased in activity.

The idea of a nuclear receptive substance is further supported by the fact that the myo-neural junction is the seat of fatigue. When a nerve is stimulated with a tetanizing current the muscle soon fails further to respond to nerve impulses; when stimulated directly, however, it is found quite capable of contraction. It is generally held that the fatigue brought on in this way is occasioned by an accumulation of the waste products which "congests" the myo-neural junction, and renders it for the time being incapable of receiving nerve impulses. Lactic acid is well known to be one of the chief substances produced by muscular activity, and the lactic acid must be oxidized to enable the muscle to continue its activity. Since it is widely agreed that the nucleus of any cell is a center of oxidation (28), it follows that the muscle nucleus would be the first of the cell elements to be affected by an increase in lactic acid; with continued stimulation the nucleus must literally become overwhelmed with material to be oxidized; as a result it would become fatigued, and would be no longer capable of performing its normal functions.

The writer is well aware of the many difficulties involved regarding muscle nuclei as the junctional tissue, and fails in his purpose if, at any point, he appears to be oblivious to them. In the first place, many will find it difficult to abandon the conception of transmission and contraction as being fundamentally cytoplasmic processes; in response to objections raised on this point, I think it can be said justly that the theory does not in reality require one to abandon such a fundamental conception, but simply to modify it. Transmission is still cytoplasmic, as is also contraction, but the link between the two must be regarded as nuclear. The nucleus merely receives a cytoplasmic impulse and having received it, excites the contractile element of the muscle to activity. Though this explanation may not prove satisfactory to some, it must inevitably be accepted if the theory is to stand.

Perhaps the most vulnerable part of the argument lies in the interpretation placed upon the staining results. Novocaine, it may be urged, might act on elements of a tissue in which it fails to become strongly concentrated; hence the tissues which stain lightly with novocaine brown may be as much affected as those which take a heavy stain. Experimentally this is perhaps the most difficult to meet of all of the objections. However, it seems much more probable that novocaine acts upon that portion of the tissue in which it is most highly concentrated. In fact the entire Ehrlich theory of physiological action is based upon this tacit assumption, and those who object must, I

think, bear the burden of the proof. If it can be shown that novocaine does not act upon the substance for which it has the greatest affinity, all of the deductions drawn from the results of staining immediately fall to the ground.

If further experimentation proves definitely the thesis herein set forth, that the synapse between nerve and muscle is one in which nuclei are concerned, it seems desirable that the function of the nuclei in the spinal synapses should be thoroughly re-investigated with this possibility in view. While the writer does not wish at present to suggest with any conviction that the "synaptic membrane" may be synonymous with nuclear membrane, investigations are now being carried on to ascertain whether or not such is the case. The seat of action of adrenalin is also being investigated.

CONCLUSIONS

The purpose of the present work was to study the nature of the physiological activity of the local anesthetic, novocaine, by application of Ehrlich's theory of drug action. The following are the chief results obtained.

A. Chemical: 1. Novocaine may be linked chemically (diazotized) with a large number of staining bases, and the compounds resulting from such a linkage, when soluble in water, possess the same physiological action as novocaine.

2. The most successful stain, made in this way, was formed by the diazotization of novocaine with *meta*-phenylenediamine. For convenience, this dye has been called "novocaine brown."

3. It may be used histologically as a prepared stain, or it may be diazotized directly into tissue (living or fixed) by dipping the tissue successively into novocaine, nitrous acid, and *meta*-phenylenediamine (washing after treatment with each reagent).

4. Used in either way, novocaine brown stains only the nuclei of tissues, never the nerve fibers, nor the non-nuclear part of the end-plates.

B. Physiological: 1. Applied to sciatic-gastrocnemius preparations, novocaine acts in a way similar to curare, since it does not interfere with nerve conduction nor with muscular contractility, but only prevents the reception by the muscle of nerve impulses. It acts, therefore, on the "receptive substance" of the muscle.

2. By intra-vital diazotization of the novocaine it is shown that the seat of action of the novocaine is in the muscle nuclei.

3. It is, therefore, suggested that the receptive substance of the muscle resides within nuclei of the muscle fibers. This is strongly corroborated by the fact that motor nerves terminate beside the end-plate nuclei—nuclei which have migrated during development from the interior of the muscle fiber.

4. Novocaine does not materially affect the rate of pulsation of chick hearts either before or after innervation.

5. Novocaine nullifies in the clam the "primary inhibition" (Göthlin) of the cilia produced by an electric current, apparently as a result of its action upon the nuclear constituents of the mantle. It seems highly probable, therefore, that the nucleus of a ciliated cell is intimately concerned in the beating of the cilia.

The investigations embodied in the present paper were commenced at the Bermuda Biological Station for Research during the summer of 1920, and were continued in the fall of that year as a research problem in Zoölogy 14,[a] a course given in the first semester by Dr. G. H. Parker. During the second semester of the college year the work was carried on as a research course under Dr. E. L. Mark.

It is with pleasure that I express my indebtedness to Doctor Parker and to Doctor Mark for their encouragement and for the many helpful suggestions which they have given me in the course of the experimental work; also to Dr. J. B. Conant for his advice concerning some of the chemical possibilities involved in the diazotization of novocaine. I wish also to thank Dr. Oliver Kamm, of the Parke-Davis laboratories, for his courtesy in supplying me with many of the drugs used in this investigation.

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TYPES OF OSCILLATIONS IN DIAPHRAGM MUSCLE

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Mosso (1) in 1886 described oscillations that appear in diaphragm muscle in rabbits and dogs under anesthesia and in normal sleep. Since that time they have been observed by many workers (2). In a series of investigations carried on in this laboratory these oscillations have been observed in urethanized cats, dogs and rabbits and in decerebrate cats and rabbits (3). The purpose of this investigation was to determine the types of forms these oscillations may take.

Methods. The animals used were dogs, cats and rabbits. One series of dogs, cats and rabbits was anesthetised with urethane (2 grams per kilo of body weight) by stomach. The other series consisted of cats and rabbits which were decerebrated under ether. Most of the observations, however, were made under urethane because of its uniform anesthetic action. An animal was fastened back downward with thongs to an animal holder, a cannula inserted into a femoral artery and a mercury manometer attached for recording the blood pressure.

A median incision was made through the abdominal wall and an S-shaped hook attached to the diaphragm about midway between the central tendon and the lateral chest wall. From the S-shaped hook a thread was passed over a pulley to a light writing lever which recorded movements of the diaphragm on a revolving drum simultaneously with the record of the blood pressure.

In the early experiments a simultaneous record was obtained of the movements of the chest wall along with that of the diaphragm muscle but it was found either to move synchronously with the diaphragm or not to move at all. The chest record was omitted in the later experiments. Also attempts were made to record by means of a tambour but this method did not prove sufficiently delicate for the small oscillations.

Continuous observations were made on these animals for periods varying from 6 to 10 hours. Inspirations are represented by down

strokes of the muscle lever and expirations by the up strokes. The temperature of the animal was maintained by means of an electric heater.

Results. In considering the results of this work it should be stated that the oscillatory rhythms appeared most prominently in the diaphragm muscle of vigorous animals, yet they failed to appear in some

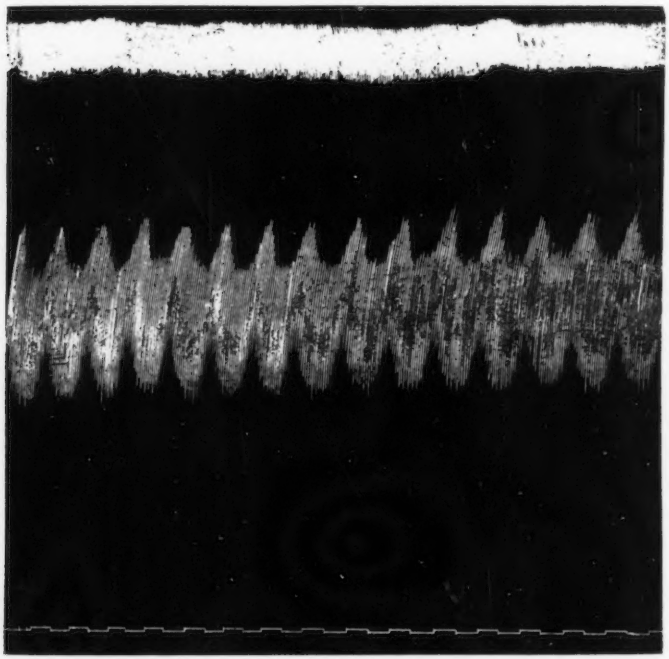

Fig 1. Dog. The upper curve indicates the blood pressure; the middle curve, contractions of the diaphragm muscle; and the lower line, the time at 5 second intervals.

that seemed normal. Another fact of general interest is that these oscillations may appear at any time from within 1 to 6 hours after the observations are begun.

Types of oscillations. Figure 1 shows a type of curve that appeared in a dog about one hour after the experiment had begun. It is seen that these oscillations occur both at the bottom and the top of the curve

and the amplitude is nearly uniform in all phases. Since the down strokes represent inspirations it indicates that the chest cavity is larger and would hold more air when the oscillations appear at the bottom of the curve, and vice versa when they appear at the top.

Figure 2 which was obtained from the diaphragm of a decerebrate cat shows a variation from the type in figure 1. The oscillations are somewhat greater at the bottom of the curve than at the top, the amplitude is not uniform and wide excursions of the lever occur from time to time as a result of gasps.

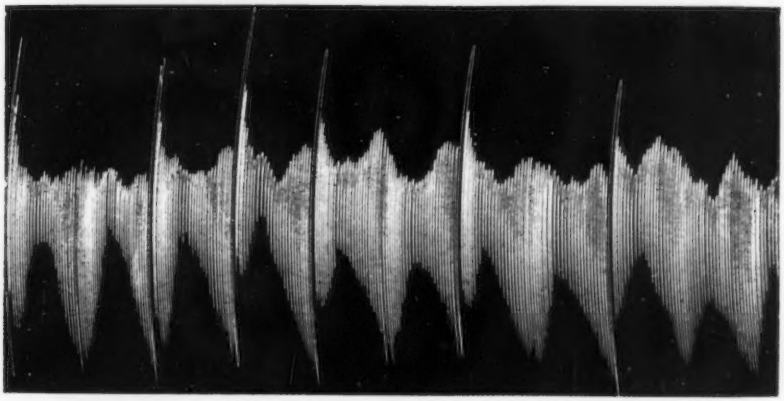

Fig. 2. Decerebrate cat. Oscillations irregular in amplitude and greater on the down strokes of the lever.

In figure 3 the oscillations take on an entirely different form inasmuch as the rhythms appear only at the top of the curve and indicate a decrease in the tonicity of the diaphragm. These oscillations appeared about 6 hours after the beginning of the observations and continued for 5 hours except for intermissions of 1 to 10 minutes which occurred from time to time until the experiment was terminated. During the course of the experiment the oscillations appeared at fairly regular intervals, then would disappear for a minute or more to reappear singly, in pairs or in trios, and then disappear and reappear later in the same manner. As the experiment proceeded the height of the oscillations became smaller.

A fourth type of curve is represented in figure 4A where the oscillatory rhythms appear at the bottom of the curve. This is a fairly com-

mon type. In this animal these oscillations appeared and then disappeared and then about 30 minutes later they had entirely changed their form and appeared at the top of the curve instead of the bottom as shown in figure 4B.

Fig. 3. Cat. Oscillations at top of curve. Time, 5 seconds

A fifth type of curve observed in these experiments is represented in figure 5, which shows compound oscillations. It is seen that large oscillatory waves appear in the diaphragm curve about every 2 to 3 minutes. These large rhythms first occurred nearly 5 hours after

the experiment was started. Another significant fact about this curve is that small oscillations are superimposed onto the large ones, especially on the right half of the curve giving compound oscillations.

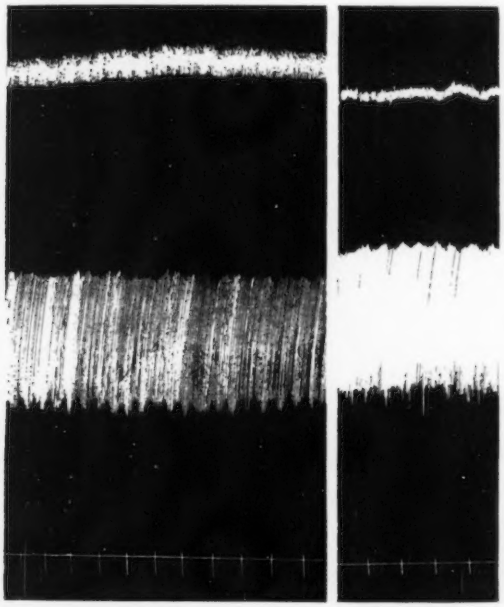

Fig. 4. Cat. Oscillations at bottom of curve in *A* and at top of curve in *B*. Time, half-minutes.

DISCUSSION OF RESULTS

Relation to blood pressure. On examining these curves it is seen that there is no direct relation between oscillatory waves of the diaphragm and changes in the blood pressure. This has been our uniform experience with dogs and cats. In the rabbit, however, we have observed a direct relation of blood pressure to oscillations in the diaphragm in some of our experiments, similar to those described by Mosso (4).

Source of oscillations. The oscillations in diaphragm muscle are usually considered to be due to influences arising from central origin (5). It is well known that changes in the carbon dioxide content of the blood

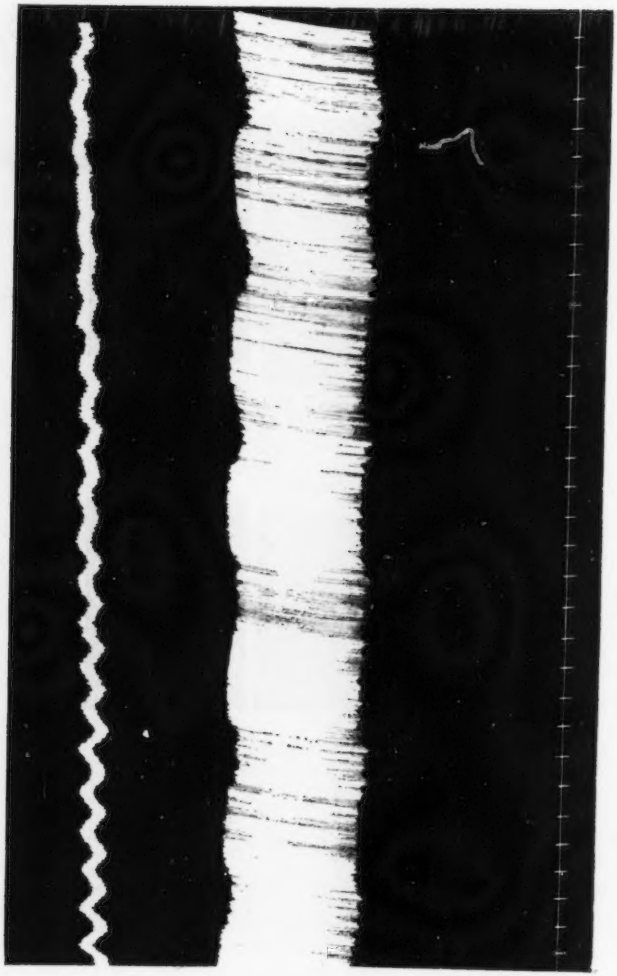

Fig. 5. Cat. Compound oscillations of diaphragm muscle. Time, half-minutes

will influence the depth of respiration. We have tried experimentally to evoke these rhythms by administering CO_2 by way of the lungs, but without success. Mosso observed oscillatory rhythms in the diaphragm and chest muscles of the dog after apnoea, which was produced by artificial respiration (5). Macleod in a recent paper has shown that "periodic breathing" may occur in decerebrate cats when the oxygen supply is reduced and that on administering oxygen the breathing returns to normal (6). In decerebrate cats and rabbits and in urethanized dogs and cats we have had oscillations appear in the diaphragm muscle when the excursions of the writing lever were greatest, indicating increased ventilation, and disappear when they were least, and vice versa. We have also had oscillations appear as in figure 3, when there are wide excursions of the lever and disappear, then later reappear and disappear when the rate of breathing is slower and the amplitude of the curve not more than two-thirds as high. This would seem to indicate that other factors besides oxgyen may influence these rhythms.

Recently we have shown that oscillations may appear in diaphragm muscle *in situ* when the medulla is pithed and the phrenic and vagi nerves are severed and the peripheral end of a phrenic nerve is stimulated rhythmically with uniform break induction shocks (7). Oscillatory rhythms have also been obtained in a similar way on various other muscles (8). Lee, Guenther and Meleney found "rhythmicity" appearing in isolated diaphragm muscle of the cat when stimulated with induction shocks at a uniform rate (9).

The fact that oscillatory rhythms may occur in diaphragm muscle when isolated from the respiratory center and stimulated with break induction shocks through a peripheral end of a phrenic nerve suggests the possibility that the oscillations appearing when the nervous system is intact may be due in part to changes in the irritability of the muscle itself.

We have found no direct indications that the oscillations in diaphragm muscle are due to fatigue. They may appear soon after the observations are begun or several hours later.

SUMMARY

1. Five types of oscillations that may appear in the diaphragm muscle of urethanized and decerebrate animals are presented.

2. These oscillations may take different forms and they may appear, disappear and reappear at any time within 1 to 6 hours after the observations are begun.

3. In general there seems to be no direct relation between the character of the oscillations in diaphragm muscle and changes in the blood pressure.

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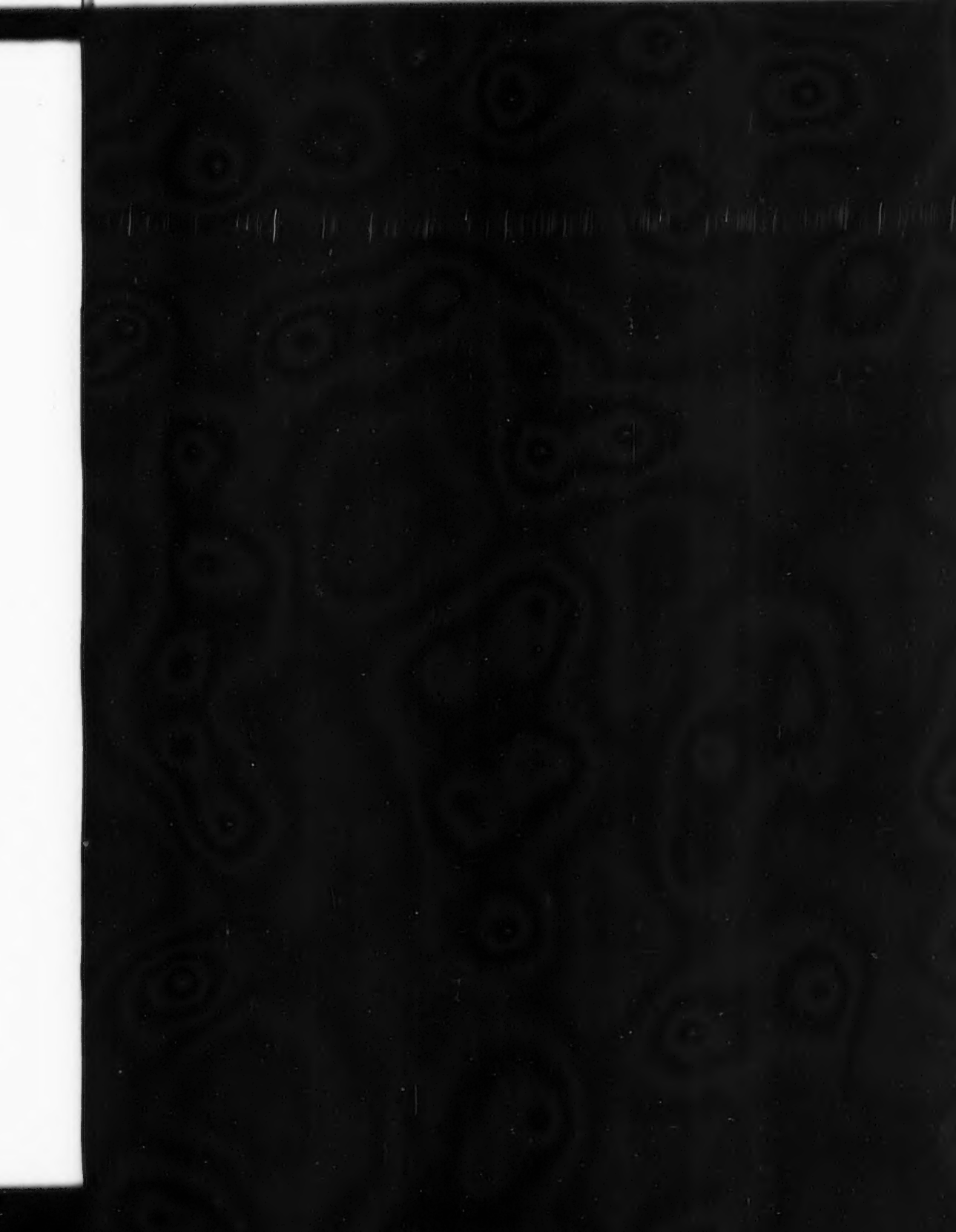
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